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Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

http://cjfas.nrc.ca

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ISSN 0706-652X / CJFSDX 62(10) 2177–2423 (2005)

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Volume 62, Number 10, October 2005

Canadian Journal of Fisheries and Aquatic Sciences

Published by the National Research Council of Canada

Volume 62, numéro 10, octobre 2005

Journal canadien des sciences halieutiques et aquatiques

Publié par le Conseil national de recherches du Canada

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Food web alterations that promote native species: the recovery of cisco (*Coregonus artedi*) populations through management of native piscivores

Damon M. Krueger and Thomas R. Hrabik

Abstract: We evaluated the effects of fisheries management on food webs in three northern Wisconsin lakes with exotic rainbow smelt (*Osmerus mordax*). In two of the lakes, restrictions on fishing reduced mortality rates on adult walleye (*Sander vitreus*) during the study period. In these lakes, walleye populations increased concurrently with a decline in rainbow smelt populations. As rainbow smelt populations declined in both lakes, native cisco (*Coregonus artedi*) populations increased. Our analysis of walleye diets illustrated that walleye fed selectively on rainbow smelt but did not feed on cisco during the summer months. When entered into bioenergetics simulations, this information demonstrates that walleye predation alone was enough to cause the observed rainbow smelt declines in our study lakes. Our results indicate that increased walleye density allows for a parallel increase in cisco density. Based on our results, fishery regulations to restore walleye to high densities in lakes invaded by rainbow smelt may restore native planktivores that have co-evolved traits.

Résumé : Nous avons examiné les effets de la gestion des pêches sur les réseaux alimentaires dans trois lacs du nord du Wisconsin contenant des éperlans arc-en-ciel (*Osmerus mordax*) exotiques. Dans deux des lacs, la restriction de la pêche a entraîné une réduction des taux de mortalité chez les dorés (*Sander vitreus*) adultes durant la période d'étude. Dans ces lacs, les populations de dorés se sont accrues parallèlement à un déclin des populations d'éperlans arc-en-ciel. Au fur et à mesure que les populations d'éperlans arc-en-ciel ont diminué dans les deux lacs, les populations indigènes de ciscos de lac (*Coregonus artedi*) ont augmenté. Notre analyse du régime alimentaire des dorés montre que durant les mois d'été les dorés se nourrissent préférentiellement d'éperlans arc-en-ciel, mais non de ciscos de lac. Cette information, incorporée aux simulations bioénergétiques, démontre que la seule prédation par les dorés ne suffit pas à expliquer le déclin des populations d'éperlans arc-en-ciel observé dans nos lacs d'étude. Nos résultats indiquent que la densité accrue de dorés permet un accroissement en parallèle de la densité des ciscos de lac. D'après ces résultats, les règlements de pêche qui visent la restauration des fortes densités de dorés dans les lacs envahis par l'éperlan arc-en-ciel peuvent rétablir les planctonophages indigènes qui possèdent des caractéristiques coévoluées.

[Traduit par la Rédaction]

Introduction

Food web manipulation offers a mechanism to manage aquatic ecosystems that have been invaded by exotic species. Within this context, predation by piscivores represents an agent that may prove useful for controlling unwanted prey fish species. However, commercial or sport fishing interests often exploit predator populations, decreasing their abundance and effects on prey species. In fact, many of the world's fisheries are overexploited (Myers and Worm 2003), and decreased predator populations have caused substantial changes in food web characteristics (Cox et al. 2002).

In some cases, decreased predator densities caused by fishing may allow less desirable fish species to dominate. For example, exotic forage fish, such as rainbow smelt (*Osmerus mordax*) and alewife (*Alosa pseudoharengus*), dominated the Lake Michigan food web after piscivore populations declined (Kitchell and Crowder 1986). This may have caused the decline of one or more commercially important native species (Crowder 1980).

Received 29 June 2004. Accepted 24 April 2005. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on 16 September 2005.
J18199

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 doi: 10.1139/F05-132 © 2005 NRC Canada

Exotic rainbow smelt are rapidly colonizing small lakes in the upper midwest and western reservoirs (Jones et al. 1994; Johnson and Goettl 1999). In many ecosystems in which rainbow smelt became established, similar patterns of negative effects were observed (Franzin et al. 1994; Hrabik et al. 1998; Hrabik and Magnuson 1999). Native coregonid species, such as cisco (*Coregonus artedi*) and lake whitefish (*Coregonus clupeaformis*), show particularly rapid declines following rainbow smelt establishment in many small northern temperate lakes (Loftus and Hulsman 1986; Evans and Loftus 1987; Hrabik et al. 1998). Therefore the future status of native coregonine populations in the upper midwest will likely be related to the range expansion and success of rainbow smelt.

Restoring or recovering native piscivore populations may provide a mechanism to control harmful exotic fish species and promote the recovery of native planktivores. In the Laurentian Great Lakes, decreases in predator abundance in response to commercial exploitation and sea lamprey predation led to increases in exotic alewife and rainbow smelt populations (Smith 1968; Wilberg et al. 2002). However, an intensive predator stocking program increased consumption of exotic prey fishes, which eventually led to decreased rainbow smelt and alewife abundance in Lake Michigan (Kitchell and Crowder 1986). Furthermore, recent changes in the fish community of Lake Superior suggest that increased predation by lake trout is associated with rainbow smelt population declines and may have facilitated reciprocal increases in native coregonine species (Hansen 1994).

Five lakes in the Northern Highland Lakes District of Wisconsin offer the opportunity to examine the influence of predator density on exotic rainbow smelt populations. Two lakes support walleye (*Sander vitreus*) populations that have increased as a result of more stringent fishing regulations and extensive stocking. A third lake contains rainbow smelt but few predators, and represents a control system that allows us to assess a rainbow smelt population in a predator depauperate environment. Data from this control lake also demonstrate the negative effect of rainbow smelt on native planktivores. In the lakes with walleye, the smelt populations declined concurrently with reciprocal increases in native cisco populations. However, the two rainbow smelt populations declined at different rates. The fourth and fifth lakes contain native piscivores and native cisco populations and allow us to determine whether cisco populations recently increased owing to changes in local climate. Our objective was to determine the cause of the observed changes in the rainbow smelt and cisco populations that occurred concomitantly with increased predator abundance in the lakes in which fishing regulations changed. The different rates of decline in rainbow smelt population sizes and subsequent increases in cisco population sizes offer insight into the influence of predators on rainbow smelt and cisco interactions. Long-term changes in prey biomass due to changes in predation rates measure the influence of a predator on a prey community (Beisner et al. 2003). We therefore hypothesized that selective predation by walleye reduced rainbow smelt abundance and led to compensatory increases in cisco populations. To evaluate this hypothesis, we used data from field studies to describe fish distribution and abundance in the five lakes from 1981 to 2002. We analyzed walleye diets and modeled fish consumption to estimate the interaction strength among walleye, cisco, and rainbow smelt in two of the lakes. We also compiled information on fishery management within the study lakes because harvest has a strong influence on predator mortality (Cox and Walters 2002; Post et al. 2002). We sought to determine whether walleye fishery restrictions led to increased walleye predation on rainbow smelt and allowed for cisco recovery. Therefore we examined the following: (*i*) correlations among population characteristics of walleye, cisco, and rainbow smelt; (*ii*) walleye diet composition throughout the open-water season to identify whether walleye preyed selectively on rainbow smelt; and (*iii*) walleye consumption rates on rainbow smelt estimated using bioenergetics models.

Materials and methods

Study sites

We studied five lakes in the Northern Highland Lakes District in Vilas County, Wisconsin (Fig. 1) from 1981 to 2002. Fence and Crawling Stone lakes are under the jurisdiction of the Lac Du Flambeau Band of the Lake Superior Chippewa Natural Resource Department, while Crystal, Trout, and Big Muskellunge lakes are under the jurisdiction of the Wisconsin Department of Natural Resources (WDNR; see Table 1 for the physical characteristics of study lakes). Trout, Big Muskellunge, Fence, and Crawling Stone lakes support popular sport fisheries and sustain relatively high densities of walleye that are augmented by annual stocking. Crystal Lake contains few piscivorous fish and is not regularly stocked.

Prior to 1989, the Lac Du Flambeau tribal council maintained liberal fishing regulations on Fence and Crawling Stone lakes. Both lakes had no minimum length limit, and non-tribal anglers were allowed to harvest five walleye per day. In 1990, the WDNR implemented a statewide 15-in (1 inch = 2.5 cm) minimum length limit on walleye. Regulations on Fence and Crawling Stone lakes became more conservative in 1997 when tribal fishery managers imposed an 18-in minimum length limit and a bag limit of three walleye per day. In addition to sport fishing, Fence and Crawling Stone lakes experienced unregulated spear fishing on walleye prior to 1997. From 1986 to 1997, spear fishers removed thousands of adult walleye each year (L. Wawronowicz, P.O. Box 67, Lac Du Flambeau, WI 54538, USA, personal communication). In 1997, the Lac Du Flambeau tribal council banned spear fishing (with the exception of tribal youth spearing in 2002) to allow the walleye populations in each lake to recover and to support egg collections for local hatchery operations.

Population density and dynamics

Fish collection

We collected walleye, cisco, and rainbow smelt using a variety of methods in Fence and Crawling Stone lakes. In the spring of 2002, we set 1.5 m square mouth, 5 mm mesh fyke nets to capture walleye during the spawning period at known spawning shoals and reefs. We removed walleye from the nets daily for 15 consecutive days on each lake. Nets were not moved during the spawning period. After spawning activity ended, we used a boom-style AC electrofishing boat to collect walleye at night from near-shore areas of both

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Fig. 1. Map of northern Wisconsin, USA, showing the locations of (*a*) Crawling Stone Lake (89°53′4″ W, 45°56′25″ N); (*b*) Fence Lake (89°50′22″ W, 45°56′56″ N); (*c*) Crystal Lake (89°36′43″ W, 46°0′5″ N); (*d*) Big Muskellunge Lake (89°37′22″ W, 46°0′9″ N); and (*e*) Trout Lake (89°40′9″ W, 46°1′9″ N).

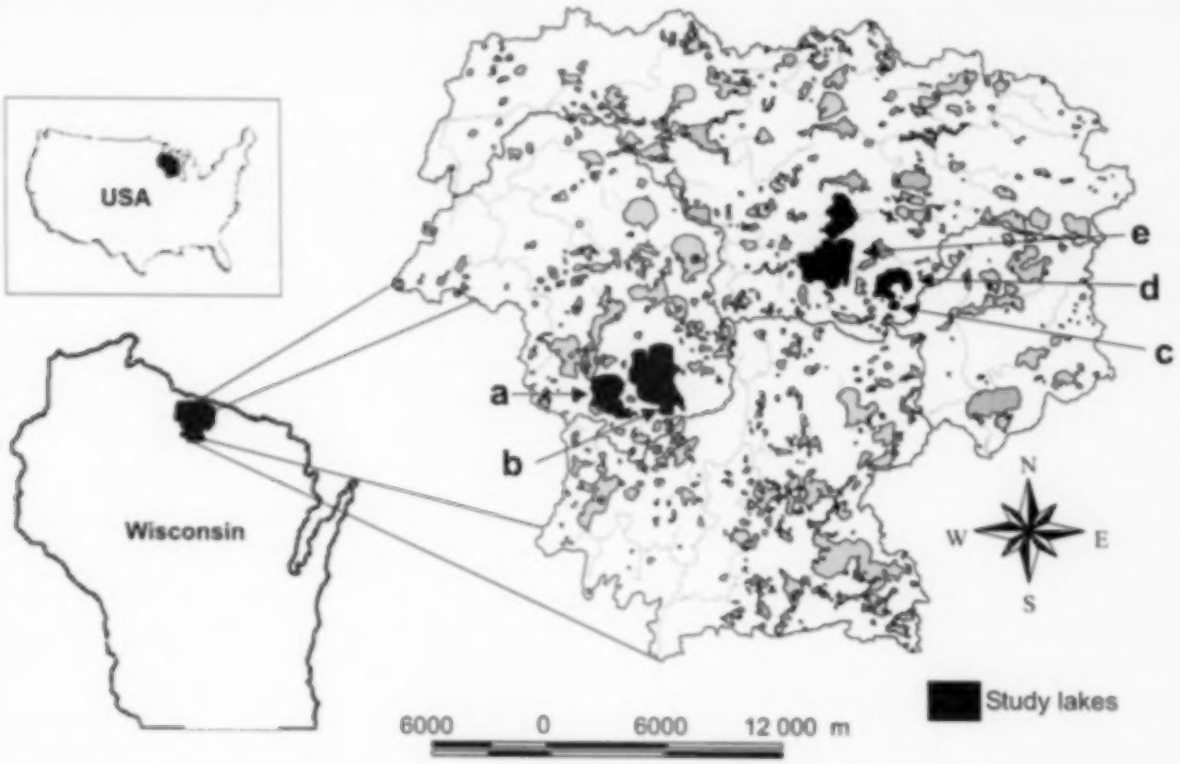

Table 1. Physical characteristics of the five study lakes likely to influence smelt colonization and viability.

Lake	Lake type	Lake area (ha)	Maximum depth (m)	Area > 6 m (%)
Fence	Drainage	1438	31	0.7
Crawling Stone	Drainage	593	24	0.6
Crystal	Seepage	46	20.5	0.6
Trout	Drainage	1544	38	0.76
Big Muskellunge	Seepage	376	21	0.56

Note: Thermocline depth was 6 m in Fence, Crawling Stone, and Crystal lakes in 2002.

lakes and to quantify the littoral zone prey fish community. We fished two 1-km transects weekly on Fence and Crawling Stone lakes from 21 May through 27 June 2002. We rotated among five sites on each lake. Following thermal stratification, we used 30 m × 3 m vertical gillnets with mesh size measuring 19, 25, 32, 38, 54, 69, 89, and 127 mm to capture pelagic fishes in Fence and Crawling Stone lakes. Nets were set monthly and biweekly throughout the summers of 2001 and 2002, respectively, to measure the abundances and sizes of pelagic forage fishes and to recover walleye diets. We set gillnets at night for less than 6 h to minimize digestion of walleye stomach contents and to maximize the number of fish caught. Using gillnet catch data, we quantified the thermal overlap among fish species (e.g., see Schoener 1970; Hrabik et al. 1998). The Long-Term Ecological Research – North Temperate Lakes (LTER–NTL) project sampled fish in Crystal Lake annually (see Magnuson et al. 1984, 1994) using fyke nets, trammel nets, and a size spectrum of vertical gillnets ranging from 19 to 89 mm stretch mesh.

Throughout the summer of 2002, length and weight information was continually collected on all fishes, and scales were collected from a subsample of each species for age estimation. Each subsample was composed of two fish for every 5-mm increment in total length. We removed at least three scales from behind the left pectoral fin in all instances.

We collected supplemental catch data for cisco and walleye in two additional LTER–NTL lakes, Trout Lake and Big Muskellunge Lake. Both lakes support sport fisheries for large piscivores, although neither supports rainbow smelt.

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Data from these lakes allowed us to track indices of cisco biomass in the absence of rainbow smelt predation but with a potential risk of predation. We also hoped to assess whether regional processes were responsible for the observed trends in cisco abundance in Fence and Crawling Stone lakes. Data were collected online via the University of Wisconsin, Center for Limnology LTER–NTL fish database.

Mark and recapture

We tagged and released walleye to obtain mark–recapture population estimates in Fence and Crawling Stone lakes using Chapman's modification of Schnabel's method (Ricker 1975). Fish were captured via electrofishing and fyke nets along the near shore areas of Fence and Crawling Stone lakes in early spring. After we marked individual walleye with T-bar floy tags, they were released in random locations, which were generally in the pelagic area of the lake. We marked 340 walleye averaging 530-mm total length from Fence Lake, and 202 walleye averaging 513-mm total length from Crawling Stone Lake.

Hydroacoustic sampling

Hydroacoustic transects were performed following lake stratification to obtain estimates of fish size, depth distribution, and density in each study lake. The LTER–NTL program used a Simrad EY-M 70 kHz single-beam ecosounder to run yearly hydroacoustic transects on Crystal Lake (three transects) from 1981 to 1994 and on Fence and Crawling Stone lakes (six and five transects, respectively) in 1993. HADAS postprocessing software was used to analyze these transects (Rudstam et al. 1993; Sanderson et al. 1999). We used a 120-kHz BioSonics DT6000 split-beam echosounder to collect hydroacoustic data in 2001 and 2002 on Fence, Crawling Stone, and Crystal lakes. We ran five transects on Fence and Crawling Stone lakes in 2001 and 2002 and three on Crystal in 2002. We used Echoview (v.3.0) software to analyze acoustic data for two nights in 2001 and four nights in 2002 on Fence and Crawling Stone lakes and one night in 2002 on Crystal Lake. Hydroacoustic estimates of smelt population density in Crystal Lake were proportional to gillnet catch rates (population estimate = 67.6 × gillnet catch per unit effort (CPUE), $r^2 = 0.95$, $p < 0.001$). We used this relationship between gillnet catch rates and hydroacoustic estimates to approximate population sizes and biomass when no acoustic data were available (1995–2000). Hydroacoustic data for Fence and Crawling Stone lakes were not available for years prior to 1993. We assumed that population densities of pelagic species were proportional to gillnet catch, as in Crystal Lake. We used 1993 gillnet catch and 1993 population estimates for Fence and Crawling Stone lakes to estimate the proportional relationships and population sizes for 1982 in Fence Lake and 1986 in both lakes.

Simrad and BioSonics echosounders were calibrated bimonthly with standard tungsten carbide spheres of known target strength. We corrected 1993 fish density estimates for bias owing to differences in target strength distributions between single and split-beam echosounders (e.g., Rudstam et al. 1999). We used fish species composition, depth distribution, and size data from vertical gillnets to categorize individual hydroacoustic targets by species. We then estimated total lake population sizes by multiplying the density of each species within 1-m depth strata with the volume of water within each stratum as in Rudstam et al. (1993) and Sanderson et al. (1999).

Analyses of body condition and fish size

Estimation of body condition

We developed lake-specific length–weight regressions to estimate variability in rainbow smelt body condition in Fence Lake (where rainbow smelt declined) and Crystal Lake (where rainbow smelt increased) between periods of high and low rainbow smelt abundance. Rainbow smelt body condition is associated with variability in their population density and offers a useful index of feeding success (Hrabik et al. 1998). We applied a linear model to log-transformed body mass and total length data pooled across years within each lake (Hrabik et al. 1998). A single regression fit to all length and weight observations across all years for a lake was used to estimate a lake-specific length–weight relationship. We grouped the residuals for individual fish by year and used the mean residual in each year as an index of the body condition of rainbow smelt in each lake. We did so to examine whether trends in smelt body condition in Fence Lake corresponded to decreases in their abundance, or whether smelt performance in Fence Lake may have been influenced by an alternative environmental variable. In addition, we sought to identify whether smelt in Crystal Lake, where predators were rare, exhibited indications of density dependence. Body condition was not calculated for Crawling Stone Lake because no rainbow smelt were captured after 1993.

Analyses of fish size

Variability in the sizes of rainbow smelt and walleye were examined to identify any changes in size that coincided with changes in abundance and mortality rates in each population. We examined the mean length of rainbow smelt in Crystal and Fence lakes and of walleye in Fence Lake through time; data points represent periods with marked differences in the abundance and mortality of each species in each lake. We expected that smelt sizes would decrease in response to increases in predation pressure by walleye in Fence Lake, and that the size of walleye would increase with decreases in their mortality.

Diet composition

We analyzed 220 walleye stomachs collected via electroshocking, gillnets, and angling in Fence and Crawling Stone lakes. Following capture, stomach contents were flushed from live fish (Seaburg 1957) and whole stomachs were excised from deceased fish caught in gillnets. We preserved stomach contents of all walleye in 95% ethanol and later separated and dried contents to obtain biomass proportions for input into bioenergetics modeling scenarios. We used numerical proportions of the common prey items to estimate prey selection using Chesson's (1983) alpha. Alpha values were calculated for individual walleye and averaged for May, June, July, and August.

Bioenergetics modeling

Fish growth

We examined scales to determine growth rates for 85 and 81 walleye from Fence and Crawling Stone lakes, respectively. We then used the proportional method (DeVries and

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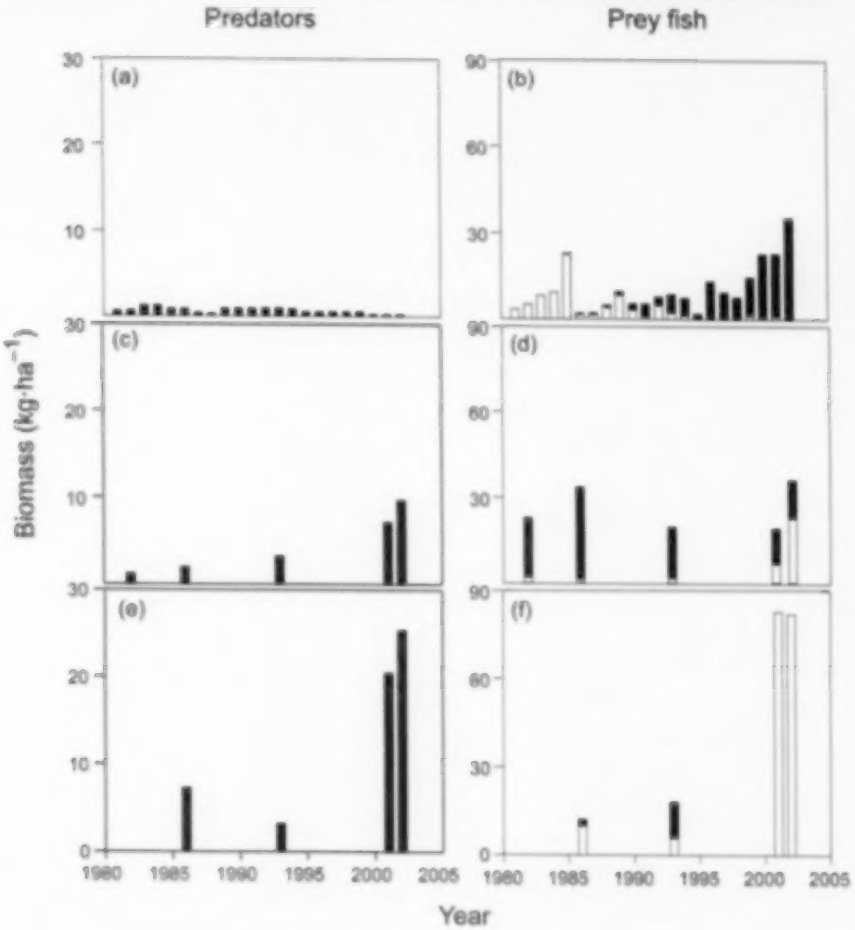
Fig. 2. The estimated biomass of predator and prey species in each study lake from 1982 to 2002. (*a*) The biomass of predatory fish in Crystal Lake represented as a combined estimate of lake trout (*Salvelinus namaycush*) and walleye (*Sander vitreus*). (*b*) The biomass of rainbow smelt (*Osmerus mordax*; solid bars) and native perch (*Perca flavescens*; open bars) in Crystal Lake.(*c*) The biomass of walleye in Fence Lake. (*d*) The biomass of rainbow smelt (solid bars) and native cisco (*Coregonus artedi*; open bars) in Fence Lake. (*e*) Walleye biomass through time in Crawling Stone Lake. (*f*) The biomass of rainbow smelt (solid bars) and native cisco (open bars) in Crawling Stone Lake. Note the difference in scale for the *y* axis between predator and prey species.



Frie 1996) to back calculate length at age for individual fish. The same method was used to estimate size at age and growth rates for rainbow smelt and cisco. Finally, we grouped individual fish into year classes according to length and averaged weights for each year class. For walleye, we also estimated the age structure of the entire population, based on the subsample of fish we aged. First, we estimated a length range for each age class. Second, we determined the proportion of sampled fish that fell into each length range. Last, we multiplied the length-at-age proportions by the acoustic population estimate to achieve a whole-lake age structure for walleye.

Temperature

We measured temperature profiles monthly during the summer of 2002 in Fence and Crawling Stone lakes. The LTER–NTL program measured temperature profiles in Crystal Lake biweekly from ice out to late November from 1981 to 2002. We estimated daily water temperature at depth in all three lakes using linear interpolation between water temperature measurement dates in each year. Temperature data were input into bioenergetics modeling scenarios.

Estimation of prey consumption

We used fish bioenergetics models to assess the potential impact of walleye predation on rainbow smelt and of rainbow smelt predation on young cisco in Fence Lake. We quantified these effects by simulating consumption by walleye and rainbow smelt with the Wisconsin bioenergetics 3.0 software (Hanson et al. 1997). In situ weight-at-age data were input as start and end points to estimate the energy budget for average individual walleye and rainbow smelt. We also input species- and depth-specific thermal data, as well as diet composition (for walleye), to estimate total annual consumption by walleye and rainbow smelt in 1982 (Fence Lake only), 1986, 1993, 2001, and 2002 in Fence and

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Crawling Stone lakes. To estimate total consumption, we first found the proportion of fish in each age class using the aforementioned methods. Then, we multiplied consumption of the average individual in each age class by the acoustic population estimate for each age class to estimate total consumption in each study year. In addition, we compared rainbow smelt biomass consumed by walleye with rainbow smelt biomass available in Fence Lake to determine the proportion of the rainbow smelt population walleye consumed in each year of the study. We obtained the caloric densities of yellow perch (*Perca flavescens*), cyprinids, and rainbow smelt used in our modeling scenarios from Bryan et al. (1996), and the caloric density of walleye from Kitchell et al. (1977).

Results

Walleye population dynamics

In Fence and Crawling Stone lakes, walleye population size and mean length increased considerably from 1982 to 2002. In Fence Lake, the adult walleye (total length > 300 mm) population density increased from 3.2 kg·ha^{-1} (1.4–5 kg·ha^{-1}) in 1993 to 9.7 kg·ha^{-1} (6.2–12 kg·ha^{-1}) in 2002 (Fig. 2). Mark–recapture estimates for walleye ranged from 3 to 42 fish·ha^{-1} (3.9–54.6 kg·ha^{-1}). Mean length of walleye increased from 361 mm in 1982 to 507 mm in 2002, indicating a significant increase in size structure (Fig. 3). Stocking of fingerling walleye averaged 28 fingerlings·ha^{-1}·year^{-1} (±12 fingerlings·ha^{-1}·year^{-1}).

In Crawling Stone Lake, the biomass of walleye also increased from 1986 to 2002. Initially, walleye biomass fell from 7.2 kg·ha^{-1} in 1986 to 3.1 kg·ha^{-1} in 1993. However, walleye biomass increased to 25.4 kg·ha^{-1} (15.2–35.4 kg·ha^{-1}) by 2002 (Fig. 2). Mark–recapture population estimates averaged 6.3 walleye·ha^{-1} and ranged from 4 to 60 walleye·ha^{-1} (4.8–72 kg·ha^{-1}) owing to low recapture rates. However, these estimates are comparable to hydroacoustic estimates. Walleye mean length was 495 mm in 2002, just short of the average in Fence Lake. Stocking of walleye fingerlings in Crawling Stone Lake was higher than in Fence Lake and averaged 44 fingerlings·ha^{-1}·year^{-1} (±17 fingerlings·ha^{-1}·year^{-1}).

In Trout Lake, catch rates of adult walleye appeared to rise slightly from 1981 to 2002. However, in Big Muskellunge Lake, there was no apparent pattern in catch rates of adult walleye (Fig. 4).

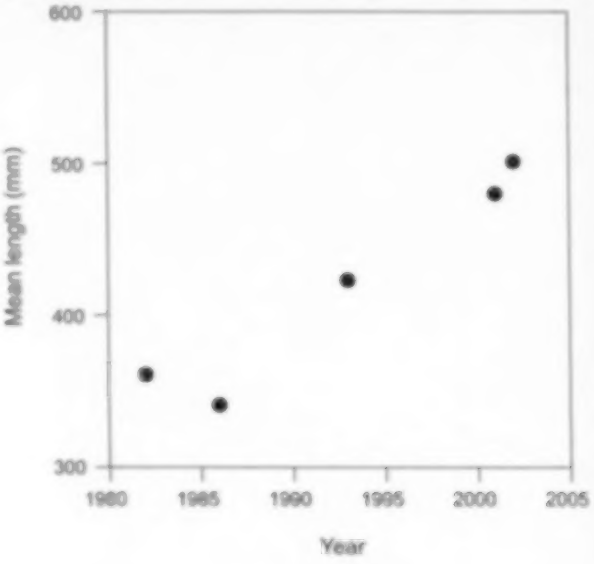
Rainbow smelt population dynamics

We observed the highest density of rainbow smelt in predator-poor Crystal Lake. The biomass of rainbow smelt increased steadily since their detection in 1986, and approached 35 kg·ha^{-1} in 2002 (Fig. 2). As rainbow smelt biomass increased after 1993, their mean length distribution did not change (Fig. 5). Rainbow smelt body condition declined after 1993 and the trend was consistent with density-dependent processes associated with increases in population size (Fig. 5). Population size of yellow perch (*Perca flavescens*), once the dominant planktivore in the lake, declined and their biomass was negatively correlated to rainbow smelt biomass (Fig. 2).

In Fence and Crawling Stone lakes, rainbow smelt biomass decreased, particularly in the latter years of our study period. In Fence Lake, rainbow smelt biomass decreased from 32 kg·ha^{-1} in 1993 to approximately 13 kg·ha^{-1} in 2002 (Fig. 2). Rainbow smelt average length also decreased through time and coincided with increases in walleye biomass (Fig. 5). In contrast, the average body condition of rainbow smelt in Fence Lake increased through time (Fig. 5). According to population age frequency data, rainbow smelt total annual mortality was 59% in 2002 (e.g., Ricker 1975).

In Crawling Stone Lake, rainbow smelt population densities increased roughly sixfold from 1986 to 1993. However, rainbow smelt population biomass in Crawling Stone Lake decreased substantially after 1993. Rainbow smelt biomass declined from approximately 12 kg·ha^{-1} in 1993 to undetectable levels by 2001 (Fig. 2). Rainbow smelt were not found in Crawling Stone Lake in 2001 or 2002, which indicates a stronger decline in rainbow smelt population density than was observed in Fence Lake.

Fig. 3. Estimates of mean size of walleye caught in Fence Lake from 1982 to 2002.



Cisco population dynamics

In Fence and Crawling Stone lakes, cisco showed tremendous increases in population size. In Fence Lake, cisco biomass increased between 1993 and 2002, and they are now the dominant pelagic planktivore in the system, both in number and by biomass. Cisco, which were common in both Fence and Crawling Stone lakes in the 1960s (S. Gilbert, Wisconsin Department of Natural Resources, 8770 Hwy J Woodruff, WI 54568, USA, personal communication), were rare in 1993 when only one individual was caught. In 1982, 1986, and 1993, no cisco younger than 4 years old were captured. Cisco abundance in Fence Lake increased substantially between 1993 and 2002. Hydroacoustic data collected in 2002 indicated that cisco biomass was approximately 23 kg·ha^{-1} in Fence Lake, a 14-fold increase from 1993 (Fig. 2). Furthermore, young fish dominated the cisco popu-

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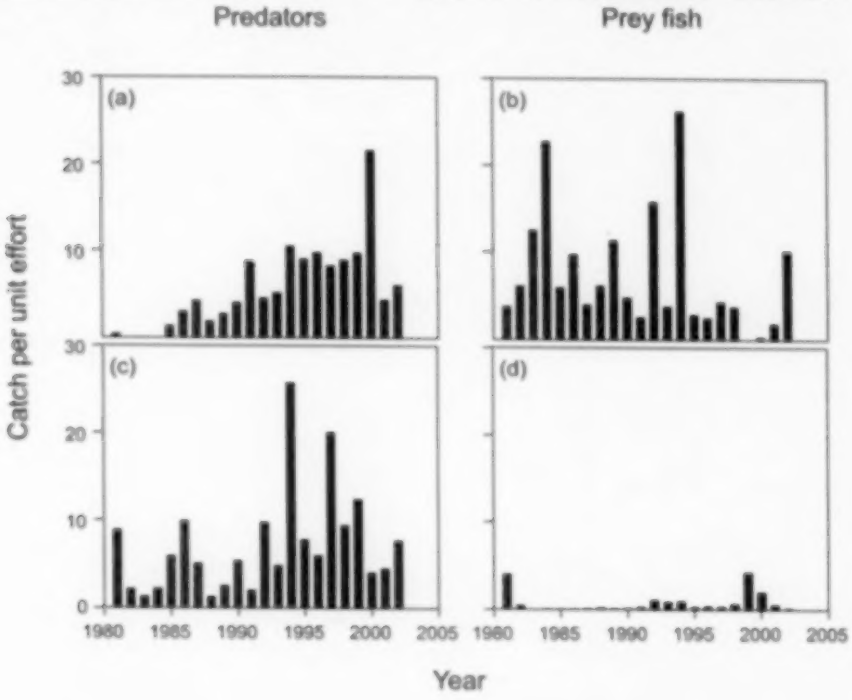
Fig. 4. Catch per unit effort (CPUE) of predator and prey species in supplemental study lakes. (*a*) CPUE of walleye (*Sander vitreus*) in Trout Lake. (*b*) CPUE of cisco (*Coregonus artedi*) in Trout Lake. (*c*) CPUE of walleye in Big Muskellunge Lake. (*d*) CPUE of cisco in Big Muskellunge Lake. Data collected by the Long-Term Ecological Research – North Temperate Lakes (LTER–NTL) program using 24-h vertical gillnet sets and three 30-min electroshocking transects (see Magnuson et al. 1984, 1994).



lation of Fence Lake in 2002, where most fish were younger than 2 years.

In Crawling Stone Lake, cisco biomass increased considerably from 1986 to 2002. Although cisco were abundant in Crawling Stone Lake in the 1960s and 1970s, their biomass declined to approximately 1.2 kg·ha^{-1} by 1993. By 2002, however, cisco biomass increased to approximately 82.5 kg·ha^{-1}. Cisco biomass was higher in Crawling Stone Lake than in Fence Lake, and fish of various ages were observed, including an abundant age-0 year class. Cisco in Fence and Crawling Stone lakes attained a length of 200 mm in their second year, a length exceeding the gape limitation of the average adult walleye in 2002, assuming walleye consume prey items that are <30% of their body length (Rudstam et al. 1993). In Trout and Big Muskellunge lakes, cisco catch rates did not consistently increase after 1993 (Fig. 4).

Walleye diet characteristics and consumption

Consumption by walleye increased throughout the study in Fence and Crawling Stone lakes, which coincided with increases in body size and population density. In 2002, Fence Lake walleye were piscivorous, and 76% of diets (n = 83 out of 110) contained fish prey. Walleye primarily consumed rainbow smelt, yellow perch, and cyprinids; other prey comprised less than 5% of the diet. In the early summer months, walleye fed mostly on cyprinids and perch and less so on rainbow smelt. In late summer, walleye fed selectively on rainbow smelt (Fig. 6), concomitantly with increasing spatial overlap between rainbow smelt and walleye (Fig. 7). Walleye did not feed on the abundant cisco in Fence Lake.

Consumption by walleye on fish prey increased substantially in Fence Lake from 1993 to 2002 and was related to increased walleye population density. Bioenergetics modeling indicated that the Fence Lake walleye population consumed approximately 55% of rainbow smelt biomass in 2002 (Fig. 8), which accounts for 93% of the total annual mortality experienced by rainbow smelt, based on population age frequencies (e.g., Ricker 1975).

Walleye in Crawling Stone Lake were less piscivorous than Fence Lake walleye in 2002 and a smaller proportion of diets contained food items (60%; n = 66 out of 110). Walleye primarily consumed ephemeropterans, yellow perch, and minnows throughout the summer. Despite the lack of rainbow smelt in gillnet samples in Crawling Stone Lake, rainbow smelt occasionally appeared in walleye diets, signifying a relict rainbow smelt population. Walleye did not feed on cisco in Crawling Stone Lake despite the relatively high abundance of the small age-0 stages of cisco.

Prey consumption by walleye in Crawling Stone Lake increased substantially from 1993 to 2002. In 2002, walleye consumed approximately 58 kg·ha^{-1}, which is roughly five times higher than the consumption rates we observed in Fence Lake (Fig. 9). We estimated that the walleye popula-

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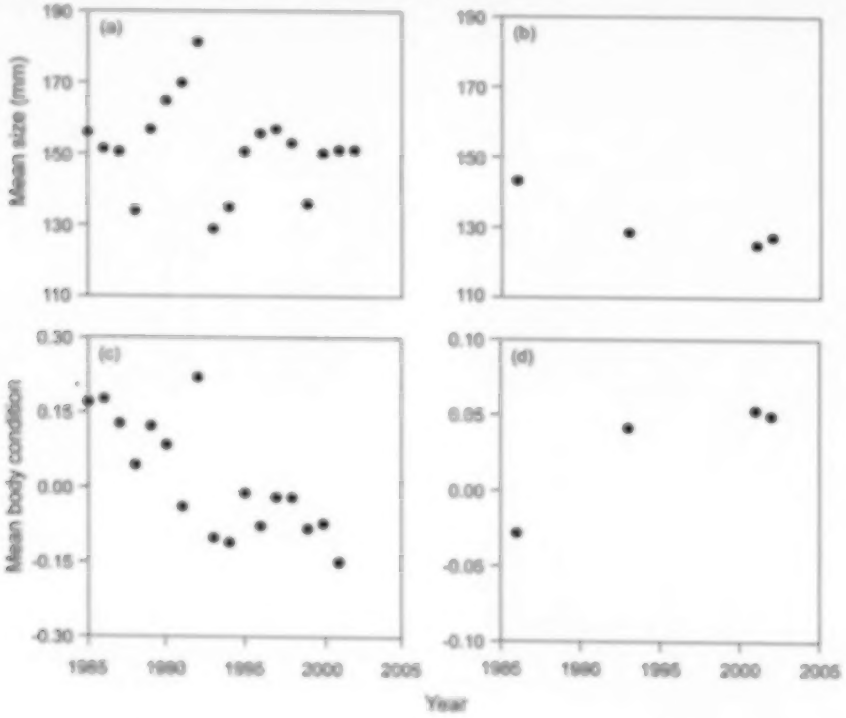
Fig. 5. The average length of rainbow smelt (*Osmerus mordax*) in (*a*) Crystal and (*b*) Fence lakes captured in gill nets and the average body condition of smelt in (*c*) Crystal and (*d*) Fence lakes from 1985 to 2002.



tion was capable of consuming approximately 72% of the adult rainbow smelt population in 1993, when rainbow smelt were last found in Crawling Stone Lake.

In Fence and Crawling Stone lakes, rainbow smelt population declines were concurrent with increases in prey consumption by walleye. Thus, there appeared to be a relationship between consumption rates by predators and rainbow smelt density. Consumption by rainbow smelt, the factor most likely associated with cisco declines, was negatively associated with consumption by piscivorous walleye in the study lakes (Fig. 10).

Discussion

Assessing food web structure and changes in rainbow smelt abundance

Manipulating the density of top predators in lake ecosystems represents a mechanism to generate changes in lower trophic levels through predator–prey interactions. These changes may ease competition–predation effects and facilitate recovery of a native species (Kitchell et al. 1988). Our observations in five northern temperate lake ecosystems that experienced rainbow smelt introductions indicate that manipulating predator communities yields results consistent with those observed in other lakes over the past few decades (*e.g.*, see Kitchell and Crowder 1986; Johnson et al. 1992; Kitchell et al. 2000). In Fence and Crawling Stone lakes, where predator stocking and predator harvest regulations facilitated increases in the native predator, the food web returned to one dominated by native species. This result was likely the effect of selective predation on exotics by native predators and species-specific characteristics.

Selective predation often alters prey community composition (Brooks and Dodson 1965; Carpenter and Kitchell 1988) and our results support this contention. Increased asymmetric predation rates in two study lakes resulted in a shift in planktivore dominance; rainbow smelt population densities declined while cisco population densities increased. Cisco biomass exceeded rainbow smelt biomass in both lakes in 2002, yet cisco were not consumed by walleye. Cisco typically represent twice the energetic benefit to a predator when compared with rainbow smelt (Bryan et al. 1996). This is counterintuitive to our finding that walleye selectively preyed upon rainbow smelt. However, our results indicate that walleye overlapped spatially with rainbow smelt more so than with cisco, particularly in late summer, and may explain the lack of cisco in walleye diets. Differences in size at age may further explain the pattern in prey preferences exhibited by walleye. All rainbow smelt were vulnerable to predation by the average-sized walleye in each lake. Cisco, however, attain a size refuge relatively quickly, making them less vulnerable to walleye predation throughout most of their lifespan. The lack of age-0 cisco in walleye diets implicates a predator avoidance behavior that may be the result of coadaptation over longer timescales. The high consumption rates exhibited by walleye on rainbow smelt in later study years (2001 and 2002) likely allowed age-0 cisco to avoid predation by rainbow smelt with greater success than in early study years (1982–1993). Our results suggest that predation by walleye substantially reduced rainbow smelt aver-

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Fig. 6. Chesson's index of prey selection for walleye (*Sander vitreus*) caught in Fence Lake in (*a*) May, (*b*) June, (*c*) July, and (*d*) August of 2002. Error bars indicate 95% confidence intervals; the dotted horizontal line in each box represents neutral selection.

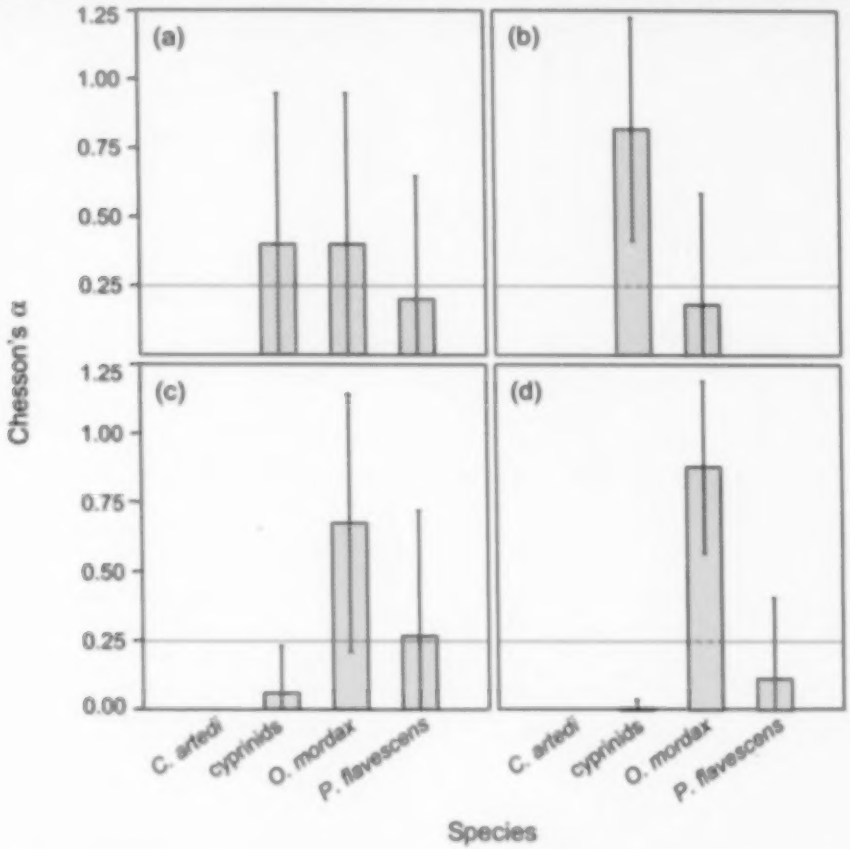


Fig. 7. Thermal overlap between walleye (*Sander vitreus*) and cisco (*Coregonus artedi*; denoted by line with solid circles) and between walleye and rainbow smelt (*Osmerus mordax*; denoted by line with ×s) as observed in vertical gillnet catch from June to August 2002 in Fence Lake.

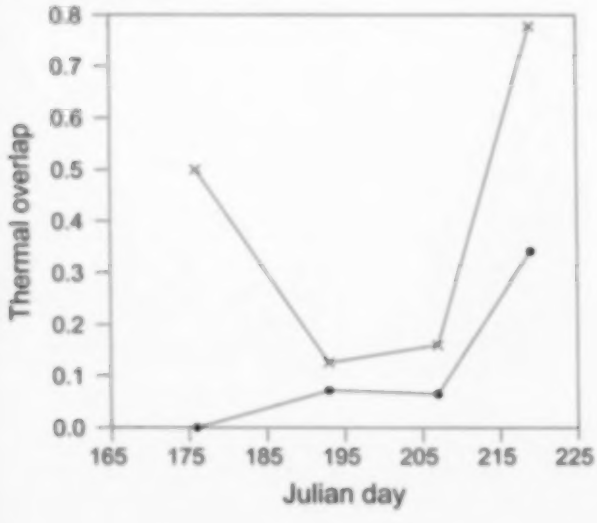
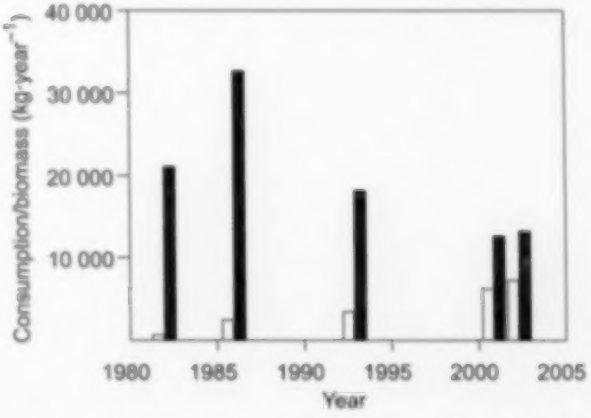


Fig. 8. Estimated consumption of smelt (*Osmerus mordax*) by walleye (*Sander vitreus*) (open bars) and estimates of smelt biomass (solid bars) in Fence Lake in each study year from 1982 to 2002.



age size, rainbow smelt population density, and consumption by the rainbow smelt populations as a whole. These declines likely reduced competition and intraguild predation rates of rainbow smelt on young cisco, and thereby decreased age-0

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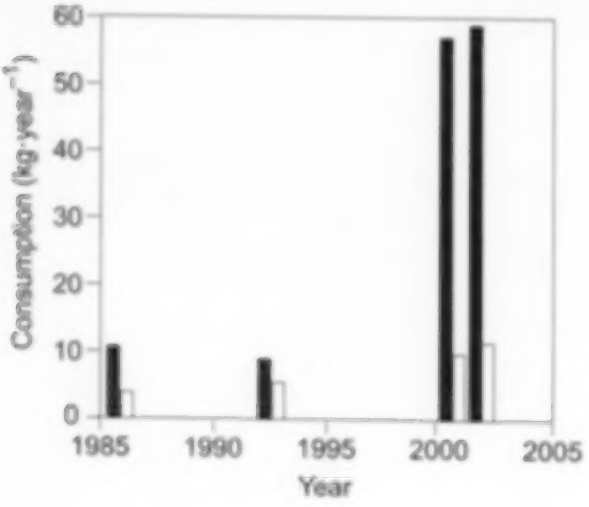
Fig. 9. Total consumption by walleye (*Sander vitreus*) in Fence (open bars) and Crawling Stone (solid bars) lakes from 1986 to 2002.

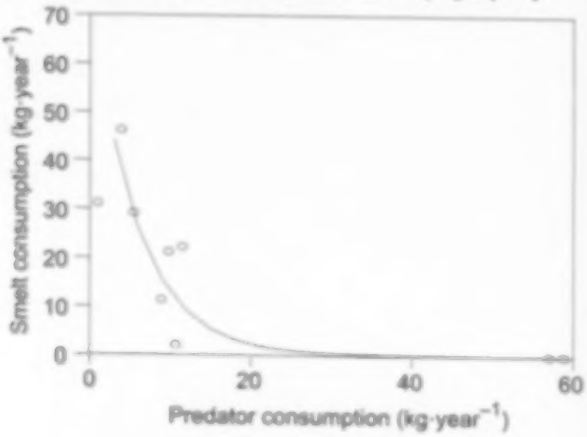


cisco mortality. These results provide strong evidence that restoring walleye populations in small lakes will not hinder the recovery of cisco. Instead, such management may lead to predator-mediated coexistence between cisco and rainbow smelt.

While predation by walleye may explain the observed declines in rainbow smelt in two of the study systems, alternative hypotheses for these declines include recruitment failure resulting from changes in regional climate, or a disease outbreak in both Fence and Crawling Stone lakes. We evaluated the first alternative hypothesis by examining population dynamics in Crystal Lake, which is an excellent control system since rainbow smelt experience very low predation rates and intraguild competition. Unlike the rainbow smelt populations in Fence and Crawling Stone lakes, Crystal Lake rainbow smelt biomass has continued to rise exponentially since they were detected in 1985. If regional processes had contributed to rainbow smelt recruitment failure in Fence and Crawling Stone lakes, such recruitment variability would likely only cause short-term fluctuations in rainbow smelt population densities (He and LaBar 1994) and would not explain the longer term patterns we observed. Therefore regional climate was not a likely underlying cause of the observed changes in rainbow smelt abundances in Fence and Crawling Stone lakes. Based on the cisco catch data from Trout and Big Muskellunge lakes, we also conclude that increases in cisco biomass in Fence and Crawling Stone lakes were not attributable to regional processes.

We cannot exclude the possibility that disease outbreak contributed to the decline in rainbow smelt. However, we did not observe any visible signs of disease in the Fence and Crawling Stone rainbow smelt populations in 2001 or 2002. In addition, rainbow smelt body condition was higher in 2001 and 2002 in Fence Lake than it was prior to 1994, suggesting a more healthy population. Our evidence is therefore most consistent with the hypothesis that selective predation

Fig. 10. The apparent relationship between consumption by walleye (*Sander vitreus*) and consumption by smelt (*Osmerus mordax*) in Fence and Crawling Stone lakes (open circles represent observed values). The solid line represents the model $SC = 70 \times e^{-0.18WC}$, where SC is consumption by smelt and WC is consumption by walleye ($p < 0.001$, $r^2 = 0.76$). The y intercept was fixed at the estimated consumption rate observed in 2002 in Crystal Lake, where smelt existed in the virtual absence of predators and where they were likely near their carrying capacity.



by walleye caused the observed declines in rainbow smelt population densities in Fence and Crawling Stone lakes.

Management implications

Managing predatory fish populations may yield several possible outcomes, including the potential control of harmful exotic species. Our study lakes represent three distinct scenarios that are defined by the level of consumption by predatory species. Predator-rich Crawling Stone Lake showed the most striking decline in rainbow smelt density. However, the extremely high density of predators in this system, coupled with the decrease in their prey, may lead to depressed growth and recruitment through density-dependent factors (Kitchell and Crowder 1986). This scenario may be acceptable if local management is focused on eliminating smelt in an effort to fully restore declining native planktivore populations. However, fishery managers may be interested in maintaining quality sport fisheries while managing for native fish populations. The intermediate walleye density represented in Fence Lake appeared to allow rainbow smelt, cisco, and walleye to coexist and maintain a diverse forage base for walleye. This situation may represent a balance between native species restoration and high predator growth rates. Jones et al. (1994) found that walleye consumed rainbow smelt almost exclusively in a Colorado reservoir, and growth increased by up to 50% in some age classes. However, the rainbow smelt population was not adversely affected by walleye predation. This was also true in Lake Champlain, where walleye and salmonid species relied heavily on rainbow smelt, but did not cause a significant decline in the rainbow smelt population (Kirn and LaBar 1996). However, in smaller oligotrophic lakes, such as Fence Lake, continued predation at current rates may further reduce rainbow smelt biomass. In Lake Michigan, rainbow smelt encountered pre-

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dation rates comparable to those in Fence Lake and subsequently declined to very low levels (Kitchell and Crowder 1986). Continued monitoring of rainbow smelt populations is needed to fully examine the sustainability of rainbow smelt under current predatory conditions in Fence and Crawling Stone lakes.

In Crystal Lake, rainbow smelt appear to show an exponential population growth pattern that has nearly eliminated the native yellow perch. Because predator abundance is extremely low, rainbow smelt will likely increase until density dependence and resource limitation lead to population declines or stabilized densities.

Our study systems represent contrasting food web configurations and resultant population dynamics. We therefore propose several options for fishery managers who face declining native fish populations caused by rainbow smelt. Management objectives should incorporate predator consumption rates on prey species as a factor when formulating management prescriptions. Our findings suggest the following: (*i*) rainbow smelt populations may decline to low levels and cisco may recover when walleye consume 58 $kg \cdot ha^{-1} \cdot year^{-1}$ of prey; (*ii*) walleye consumption rates at approximately 12 $kg \cdot ha^{-1} \cdot year^{-1}$ may promote a diverse forage base but allow native cisco to recover; and (*iii*) unchecked rainbow smelt populations will likely grow exponentially and cause native species to decline (Hrabik et al. 2001). Our results are likely specific to situations in which walleye depend on rainbow smelt for a substantial part of their diet, and the attributes of the lakes are similar to those observed in this study. Although walleye can be significant predators on rainbow smelt and alewife (Porath et al. 2003), they may not be as effective at controlling all exotic forage fishes. Ogle et al. (1996) showed that walleye did not prey upon ruffe (*Gymnocephalus cernuus*) at high enough rates to control their abundances in the western arm of Lake Superior. Changes in the food web of Fence and Crawling Stone lakes indicate that walleye predation can indirectly allow cisco populations to recover. As a result of widespread stocking of walleye by management agencies in the upper Great Lakes region, the applicability of such strategies represents a mechanism to prevent rainbow smelt establishment and restore threatened populations of cisco in their southern ranges. Fishery managers will need to identify the food web configuration and predator consumption regime that best suits the objectives for the ecosystems within their jurisdiction.

Acknowledgements

This study was performed at the University of Wisconsin, Center for Limnology, Trout Lake Research Station. Numerous individuals and entities contributed assistance throughout this study. We thank the Great Lakes Indian Fish and Wildlife Commission, the Lac Du Flambeau tribal hatchery, John Pastor at the University of Minnesota, Duluth, Greg Sass and Brian Roth at the University of Wisconsin, Madison, Tim Kratz, Pam Faschingbauer and John Vehrs at the Trout Lake Research Station, Steve Gilbert at the Woodruff, Wisconsin DNR, and Bill Swenson at the University of Wisconsin, Superior. Funding for this project was provided by the Sand County Foundation, Bradley Fund for the Environment, the North Temperate Lakes Long-Term Ecological Research Program (funded by grant DEB 0217533) and the University of Minnesota Duluth Graduate School.

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A comparison of population structure in black rockfish (*Sebastes melanops*) as determined with otolith microchemistry and microsatellite DNA

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Abstract: The black rockfish (*Sebastes melanops*) is a long-lived (≤50 years) viviparous species with an extended pelagic larval period (3–5 months) and a broad continental distribution. Prolonged larval periods have been assumed to lead to widespread dispersal resulting in minimal population structure. We tested this assumption by comparing otolith microchemistry and microsatellite DNA of adult black rockfish from four locations in Oregon and Washington. We observed significant differences among locations in element-to-Ca ratios (Mg:Ca, Mn:Ca, Zn:Ca, Sr:Ca, and Ba:Ca) at the otolith edge, which represented the last year of life. Discriminant function analysis, based on otolith element-to-Ca ratios classified, on average, 64% of the fish to collection location, suggesting that the majority of individuals from these locations did not mix. Using microsatellite DNA, we observed significant genetic differences among adults collected 340–460 km apart (F_{ST} = 0.018 ± 0.004). Using five and seven loci, 63% and 75% of the fish, respectively, were correctly assigned to collection location. These two techniques provided corroborative and complementary information on the population structure of *S. melanops*.

Résumé : Le sébaste noir (*Sebastes melanops*) est une espèce vivipare à grande longévité (≤50 ans) qui possède une période de vie larvaire pélagique prolongée (3–5 mois) et une vaste répartition continentale. On assume généralement qu'une période larvaire prolongée entraîne une vaste répartition qui mène à une structure démographique minimale. Nous avons vérifié cette supposition en comparant la microchimie des otolithes et l'ADN microsatellite des sébastes noirs à quatre sites en Oregon et au Washington. Il y a des différences significatives d'un site à l'autre des rapports des éléments sur le calcium (Mg:Ca, Mn:Ca, Zn:Ca, Sr:Ca et Ba:Ca) en bordure des otolithes, zone qui correspond à la dernière année de vie. Une analyse des fonctions discriminantes basée sur les rapports éléments/calcium classe en moyenne 64 % des poissons dans leur site de récolte correspondant, ce qui indique que la majorité des individus de ces sites ne se mêlent pas aux autres. À l'étude de l'ADN microsatellite, il y a des différences génétiques significatives (F_{ST} = 0,018 ± 0,004) entre des adultes récoltés à des distances de 340–460 km les uns des autres. En utilisant 5 et 7 locus, il est possible de classer avec succès respectivement 63 % et 75 % des poissons selon leur site de récolte. Ces deux techniques fournissent des informations complémentaires qui corroborent la structure démographique de *S. melanops*.

[Traduit par la Rédaction]

Introduction

Marine species with long larval periods are typically assumed to have widespread dispersal and thus comprise open populations that exhibit minimal population structure (Cowen et al. 2000; Palumbi 2003). This assumption, however, has been challenged by evidence of limited larval dispersal (Marliave 1986; Swearer et al. 2002; Miller and Shanks 2004) and complex spatial structure in marine fish populations (Bentzen et al. 1996; Rocha-Olivares and Vetter 1999; Taylor and Hellberg 2003). A greater understanding of marine population dynamics is needed for adequate management, particularly for the design and establishment of marine-protected areas. Yet there are relatively few methods currently available to generate empirical estimates of migration and exchange among marine populations.

Otolith microchemistry and microsatellite DNA analyses have both shown promise in investigations of spatial structure in fishes (O'Connell and Wright 1997; Campana and Thorrold 2001). The chemical composition of an otolith can be measured at discrete locations along the growth axis to provide a chronological map of elemental concentrations (e.g., magnesium, strontium, and barium) that potentially enables researchers to reconstruct aspects of a fish's environmental history (Edmonds et al. 1989; Campana et al. 1994; Campana 1999). Microsatellite DNA consists of short sequence repeats (2–5 base pairs) that are distributed throughout most eukaryotic genomes (O'Connell and Wright 1997).

Received 1 September 2004. Accepted 24 April 2005. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on 21 September 2005.
J18295

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 doi: 10.1139/F05-133 © 2005 NRC Canada

These sequences undergo relatively rapid mutation rates that lead to high levels of polymorphism and are therefore useful for fine-scale population discrimination in marine (Bentzen et al. 1996; Buonaccorsi et al. 2002), anadromous (Banks et al. 2000, 2003), and freshwater (O'Connell and Wright 1997) fishes. We are aware of only one other study that completed otolith microchemistry and genetic analyses on the same individuals even though they may provide independent yet complementary estimates of population structure. Milton and Chenery (2001) compared the results of their study using otolith microchemistry to determine population structure of the shad hilsa *Tenualosa ilisha* with the results of a genetic study using allozyme data (Salini et al. 2004). Although the studies were completed and reported separately, they each identified population structure at relatively large spatial scales (i.e., >1000 km). Because microsatellite DNA data are more useful than allozyme data for discriminating spatial structure in fish populations, the combination of otolith microchemistry and microsatellite DNA analyses may prove to be a very powerful combination for investigations of population structure in fishes.

Otolith microchemistry studies that investigate population structure typically employ one of two approaches (Thresher 1999). In the first approach, otolith elemental signatures of larvae or juveniles, which represent natal origins, are compared with those of adults collected at a later time to identify the natal origins of the adults (Thorrold et al. 2001; Patterson et al. 2004). In the other approach, individuals are collected across a relatively broad region to seek geographic groupings based on otolith elemental signatures (Thresher 1999; Campana and Thorrold 2001).

Such studies rely on two primary assumptions. The first is that otolith growth is continuous with no evidence of resorption; thus, an otolith provides a permanent chronological record. This assumption is reasonably well supported by empirical studies (Campana 1999; Thresher 1999; Campana and Thorrold 2001). Second, if fish reside in water masses with different chemical compositions and (or) temperatures, those properties will be reflected in otolith microchemistry. This assumption has yet to be fully verified because the mechanistic control of elemental incorporation is not thoroughly understood (Thresher 1999; Campana and Thorrold 2001).

Microsatellite markers, primarily because of their high levels of polymorphism, have revealed genetic structure even in marine populations with low levels of divergence (Bentzen et al. 1996; Buonaccorsi et al. 2002). Marine species often have relatively long pelagic larval periods (weeks to months) and hence high potential for long-distance dispersal (Palumbi 2003; Shanks et al. 2003). Additionally, the exchange of relatively few individuals (i.e., ≥10) can prevent the accumulation of large genetic differences among groups of fish (Slatkin 1987; Palumbi 2003). Therefore, estimates of genetic divergence among marine populations tend to be low and statistical differences are often non-significant (Waples 1998). Current limitations of the microsatellite technique include the following: (*i*) a lack of agreement on the underlying mutation model (i.e., stepwise mutation model vs. infinite allele model); (*ii*) fairly common occurrence of null alleles (i.e., alleles that fail to amplify); and (*iii*) the inability to determine whether two alleles that are identical in size are also identical by descent (Jarne and Lagoda 1996).

The *Sebastes* species complex comprises over 90 species, the majority of which are found in the Pacific Ocean. They are long lived, and at least some are viviparous (Yoklavich and Boehlert 1991; Love et al. 2002). The black rockfish (*Sebastes melanops*) ranges in distribution from the Aleutian Islands, Alaska, to southern California. Parturition (larval release) occurs from January to March (Bobko and Berkeley 2004), although release areas are unknown. Larvae spend between 3 and 6 months in the plankton (Love et al. 2002). Benthic juveniles initially settle in nearshore waters and move into deeper waters as they grow. Most individuals reach sexual maturity at between 5 and 7 years of age (Bobko and Berkeley 2004). Adults commonly occur in waters that are <55 m in depth (Laroche and Richardson 1980). Although the species has traditionally comprised a large portion of Oregon's recreational fishery, restrictions have been placed on recreational and commercial harvest to minimize impacts on Oregon populations (PMCC 1999; ODFW 2003).

Movement patterns of adult, juvenile, and larval *S. melanops* are not well understood. Mathews and Barker (1983) describe two mark–recapture studies of adults. The majority, 63% and 69%, of the tagged adults were recaptured within 300 m of their release location. Miller and Shanks (2004) observed geographically distinct otolith signatures throughout ontogeny in juvenile *S. melanops* collected 120–420 km apart. Those signatures accurately predicted an individual's collection location an average of 74% of the time, which suggests that larval and juvenile movements may be relatively limited. Combined, these data indicate that *S. melanops* may have minimal adult movements and limited larval dispersal.

Most previous studies of otolith chemistry have focused on fish collected from fresh water, estuaries, or along cross-shelf gradients (Gillanders and Kingsford 1996; Thorrold et al. 1998; Swearer et al. 2002). On the other hand, Miller and Shanks (2004) documented significant differences in the otolith chemistry of fish collected on a latitudinal gradient along the Oregon–Washington coast. Given the geographically distinct otolith elemental signatures observed in juvenile *S. melanops* (Miller and Shanks 2004) and the apparent site fidelity of adults (Mathews and Barker 1983), we determined that collections of adult *S. melanops* from locations similar to those used in the juvenile study would be ideal for a comparison of population structure as estimated with otolith microchemistry and microsatellite DNA data.

In this study, we completed both otolith microchemistry and microsatellite DNA analyses on adult *S. melanops* to examine patterns of population structure along the Oregon–Washington coast. There were five primary questions. (*i*) Does otolith elemental composition vary significantly with geographic location? (*ii*) Does otolith elemental composition provide discriminatory power to classify fish to collection location? (*iii*) Do microsatellite DNA data provide evidence of genetic structure? (*iv*) Do microsatellite DNA data provide discriminatory power to classify fish to collection location? (*v*) Are data generated with otolith microchemistry and microsatellite DNA analyses complementary?

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Fig. 1. Collection locations for adult black rockfish (*Sebastes melanops*; underlined). Fish were collected in 2001 from the following locations: north of Grays Harbor, Washington, on 3 August (n = 50); Cape Arago, Oregon, on 3 June (n = 33); Gold Beach, Oregon, on 3 July (n = 49); and Brookings, Oregon, on 16 June (n = 17). Inset shows location on the US west coast.

Methods

Fish collection

Adult black rockfish were collected from recreational fishing vessels at four locations (Grays Harbor (Washington) and Cape Arago, Gold Beach, and Brookings (Oregon)) between 3 July and 3 August 2001 (Fig. 1). Differences in coastal geology, upwelling intensity, and salinity occur along the broad (>450 km) stretch of coast represented by these sampling locations (Landry et al. 1989). Sediments from the Columbia River dominate coastal waters from Grays Harbor, Washington, to Tillamook Head, Oregon (Komar 1997; Fig. 1). Oregon's Coast Mountain Range extends from the Columbia River southward to the Klamath Mountain Range, which originates at the Coquille River (Orr et al. 1992). These ranges have volcanic rocks of different age and mineralogy that also may affect water chemistry. We included one location north of the Columbia River, one within the Oregon Coast Range, and two within the Klamath Mountain Range in an effort to maximize potential alongshore differences in water chemistry that, in turn, may affect otolith chemistry.

All fish were collected from each location on the same day. In the field, fish were measured to the nearest cm (total length, TL) and caudal fin clips (~1 cm^2) were collected and stored in 95% ethanol. Otoliths were either immediately removed (Cape Arago samples) or heads were frozen (<1 month) prior to otolith removal (all other samples). A total of 149 fish were used for microsatellite DNA analyses, 91 of which were also used for otolith microchemistry anal-

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yses. Fewer fish were analyzed for otolith microchemistry because of the relatively high cost per sample.

Otolith preparation and ageing

Sagittal otoliths were removed with acid-washed (10% Omni*Trace* Ultra™ HNO_3, VWR™, Bristol, Connecticut) plastic forceps, cleaned, weighed to the nearest 0.01 g, and measured to the nearest mm. Otoliths were cleaned ultrasonically for 15 min in NANOpure® water (Barnstead International, Dubuque, Iowa), dried in a Class 100 laminar flow hood (Envirco Corporation, Albuquerque, New Mexico), and stored in acid-washed plastic vials. Left sagittal otoliths were embedded in resin (Spurr's low viscosity resin; Electron Microscopy Sciences, Hatfield, Pennsylvania) and ground to expose the dorsal–ventral growth axis from core to edge. Otoliths were ground with 3M™ Tri-m-ite wetordry™ paper (240–1200 grit; 3M, St. Paul, Minnesota), polished with Buehler AlO_2 powder (12.5 and 3.0 µm), and again cleaned ultrasonically in NANOpure® water for 15 min. Right sagittal otoliths were stored in plastic vials for age analysis.

Formation of annual otolith increments in *S. melanops* was validated by the Committee of Age Reading Experts (C.A.R.E. 2000). Increments were visualized with the break-and-burn protocol (C.A.R.E. 2000) and a Leitz dissecting microscope at 6.5×–25× magnification. Three independent counts on 3 different days were made for each otolith, averaged, and rounded to the nearest whole year. Regression analyses were performed for fish size (TL), otolith mass, and increment number to evaluate the strength of those relationships. Analysis of covariance (ANCOVA) was used to determine if there were size-at-age differences among locations. Because of the assumption of homogeneity of slopes required for ANCOVA, comparisons of adjusted population means are also comparisons of *y* intercepts (Quinn and Keough 2002). Therefore, ANCOVA was used to compare ages, adjusted for total length, at each location. Age was the dependent variable and site was the categorical predictor, with TL as the continuous predictor.

Otolith microchemistry analysis

Otolith elemental composition was measured with a laser ablation – inductively coupled plasma mass spectrometer (LA–ICPMS). All mass spectroscopy was completed at the Oregon State University's WM Keck Collaboratory for Plasma Spectrometry, Corvallis, Oregon. A VG PQ ExCell ICPMS with a New Wave DUV193 excimer laser (New Wave Research, Fremont, California) was used for all analyses. The laser was set at 10 Hz with a 50-µm ablation spot size and a scan speed of 30 µm·s^{-1}. Skimmer cones were cleaned and gas tubing was changed daily. Background levels of calcium were monitored for accumulation in the spectrometer. No data were collected during periods with elevated background calcium levels.

Time-resolved software (PlasmaLab International, Everett, Washington) allows elemental measurements to be made at discrete positions on the otolith and integrated and averaged for each element collected. This sampling technique averages otolith element measurements over a distinct period in the life of each individual. All data were collected from linear transects within the anterior–dorsal otolith quadrant, which consistently had the clearest increments. All transects were placed toward the dorsal side of the quadrant, approximately 90° from the rostrum. For each fish, data were collected along one straight 300-µm transect that terminated at the outermost dorsal edge of the otolith. Total otolith width (at the widest point) averaged 6.3 ± 0.6 mm standard deviation (SD). The length of the last annual increment at the outer edge of the dorsal axis was, on average, 312 ± 79 µm SD. Therefore each 300-µm transect integrated over the last year of a fish's life.

Preliminary analyses indicated that the following nine analytes were consistently at concentrations well above background levels: ^{25}Mg, ^{43}Ca, ^{48}Ca, ^{55}Mn, ^{66}Zn, ^{86}Sr, ^{87}Sr, ^{138}Ba, and ^{208}Pb. Multiple transects within the anterior–dorsal quadrant were collected to determine if there were significant differences among replicate transects. No significant differences in log-transformed element concentrations were detected among triplicate transects ($n = 6$, $F \leq 0.8$, $p \geq 0.50$).

Otoliths were arranged with two (32 slides) or three (9 slides) per slide. Otoliths on the same slide were from the same collection location. Slides were acid washed prior to mounting otoliths with thermoplastic glue. Mounted otoliths were cleaned ultrasonically in NANOpure® water for 15 min, dried in a Class 100 laminar flow hood, and stored in acid-washed plastic containers for transport to the spectrometer. Mass spectroscopy was completed over a 3-day period, with an average of 15 slides randomly sampled per day. National Institute of Standards and Technology glass standards (NIST 612) were used during data collection. Data from one NIST 612 transect were collected with each otolith slide. A 45-s argon gas purge occurred before and after each transect.

Trace-element concentrations in NIST 612 slides are homogenous for all but two of the elements used in this study (Pearce et al. 1997; Eggins and Shelley 2002). Average elemental concentrations (ppm) are reported in Pearce et al. (1997). Eggins and Shelley (2002) reported some heterogeneity for certain elements, including manganese (often >10%) and lead (usually <10%). The mean relative standard deviations (%RSD) for NIST 612 glass slides during our data collection were as follows: ^{25}Mg, 10.2%; ^{43}Ca, 5.3%; ^{48}Ca, 7.4%; ^{55}Mn, 5.3%; ^{66}Zn, 7.3%; ^{86}Sr, 6.8%; ^{87}Sr, 6.0%; ^{138}Ba, 6.2%; ^{208}Pb, 6.9% ($n = 39$). Background analyte levels were determined with an argon gas blank prior to each transect and subtracted from mean otolith isotope counts. Elemental data (in counts per second) were divided by ^{43}Ca to adjust for variability in instrument sensitivity and the amount of ablated material (Campana et al. 1997). A correction factor was determined by dividing the measured NIST 612 ratios by the mean element-to-Ca ratios (ppm) reported in Pearce et al. (1997). Measured otolith element-to-calcium ratios were then multiplied by the correction factor to standardize measurements and account for instrument drift or day-to-day variations in instrument sensitivity across the atomic mass range (Jeffries 2001).

We wanted to determine if there were any effects of fish age on the otolith element-to-Ca ratios. Therefore we used a multivariate analysis of variance (MANOVA) to test for differences in element-to-Ca ratios due to fish age. Blocks (random) were represented by ages, and Mg:Ca, Mn:Ca, Zn:Ca, Sr:Ca, Ba:Ca,

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and Pb:Ca were dependent variables. Similarly, MANOVA was used to test for differences in otolith element-to-Ca ratios among the four collection locations. Blocks (random) were represented by location and the six element-to-Ca ratios were dependent variables. STATISTICA® (Version 6.0; StatSoft Inc. 2002) was used for these analyses.

Quadratic discriminant function analysis (DFA) was used to predict collection location based on otolith element-to-Ca ratios. The best validation of a DFA is the assignment of new cases (i.e., those not used in the development of the original classification algorithm). However, jackknifed predictions are used to provide a more realistic estimate of the ability of the predictor variables to separate groups when sample sizes are not large enough to generate two independent data sets. Therefore, jackknifed classifications were calculated with the removal of one observation a total of n times ($n = 91$) and averaged. SYSTAT® (Version 9.01; Systat Software Inc. 1998) was used for the DFA analyses.

Microsatellite DNA analysis

For all samples, DNA was extracted from fin clips in a 5% Chelex® (Bio-Rad Laboratories, Hercules, California) solution (Banks et al. 2000). Microsatellite DNA was amplified by polymerase chain reaction (PCR) under the following conditions: a 5-μL volume reaction contained approximately 1.5 μL of DNA template, 1× polymerase buffer (500 mmol·L^{-1} KCl, 100 mmol·L^{-1} Tris–HCL, 1% Triton® X-100), 1.5 mmol·L^{-1} $MgCl_2$, 200 μmol·L^{-1} dNTP, 0.1 μL *Taq* DNA polymerase (Promega Corp., Madison, Wisconsin), and 0.5 μmol·L^{-1} primer. Forward primers were fluorescently labeled. We used seven previously published microsatellite primers that consistently amplified and displayed polymorphism in *S. melanops*, including *Spi*4, *Spi*6, *Spi*10, *Spi*12 (Gomez-Uchida et al. 2003), *Sma*7 (Wimberger et al. 1999), *Sal*3, and *Sal*6 (Miller et al. 2000). Temperature profiles used were as reported in original primer descriptions. An MJ Research™ Peltier Thermal Cycler (Bio-Rad Laboratories) was used for all PCR reactions. Amplified fragments were separated in a MJ Research™ BaseStation using 5% polyacrylamide gels and GENESCAN® 400HD (Applied Biosystems, Foster City, California) size standards, and scored with Cartographer™ software (MJ Bioworks, Inc., Sauk City, Wisconsin).

Observed (H_{obs}) and unbiased expected heterozygosities (H_{exp}), and total (A_T) and mean (A_M) number of alleles were determined with GENETIX version 3.3 (http://www.univ-montp2.fr/~genetix/genetix/genetix.htm). Exact tests of Hardy–Weinberg equilibrium (HWE) and population differentiation were run with GENEPOP (Markov chain algorithm, 200 000 steps; Version 3.3; Raymond and Rousset 1995). We tested for gametic disequilibrium (200 permutations) and generated unbiased estimates of F statistics (F_{ST} and F_{IS}; Weir and Cockerham 1984) and G_{ST} with FSTAT (Version 2.9.3.2; Goudet 2001). F_{ST} quantifies the proportion of genetic variation among populations (Hartl and Clark 1997). F_{IS} is an estimate of the heterozygosity deficit within populations and is considered to be an indicator of inbreeding. G_{ST} is an equivalent estimate of F_{ST}, independent of sample size (Nei 1987). Error estimates for F_{ST}, F_{IS}, and G_{ST} were generated by jackknifing over all loci. Permutation tests (1000 randomizations) were used to determine if the overall F_{ST} was statistically greater than zero and if pairwise comparisons were statistically significant. All probabilities were adjusted for multiple comparisons using a sequential Bonferroni correction.

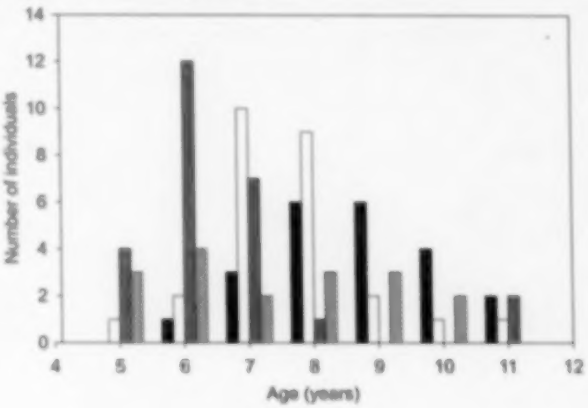

Fig. 2. Age–frequency distribution of the black rockfish (*Sebastes melanops*). Collections represented: Grays Harbor, solid bars; Cape Arago, open bars; Gold Beach, shaded bars; Brookings, cross-hatched bars.

WHICHLOCI (version 1.0) was used to predict collection location based on microsatellite data (Banks et al. 2003). WHICHLOCI resamples genotypes based on allele frequencies to generate genotypes of user-defined sizes. Genotype data for each collection location were resampled to generate sample sizes of 1000. Group membership was then predicted for 10 independent resampling events, thus generating 10 replicate classifications. This allowed average classification success using microsatellite DNA to be compared with classification success using otolith microchemistry.

Results

Otolith microstructure

Black rockfish ranged in age from 5 to 11 years. However, ages were not evenly distributed among collection locations (Fig. 2). Grays Harbor had more 9- and 10-year-olds, while Gold Beach had more 6- and 7-year-olds. TL was significantly and positively correlated with otolith mass and increment number ($r^2 > 0.70$, $p < 0.01$). ANCOVA was used to determine if there were differences in size at age among locations. The assumptions of normality, homogeneous variances, and homogeneity of slopes ($F = 1.9$, $p = 0.12$, error degrees of freedom (df) = 81) were met. There were no differences in age, adjusted for TL, among locations ($F = 0.8$, $p = 0.49$, error df = 84). We used 89 fish in the otolith microstructure analyses because we had no length measurements for two individuals.

Otolith microchemistry

The age distribution of fish among locations was skewed (Fig. 2), so all data could not be pooled in a multivariate analysis of variance to test the effect of age on otolith composition without introducing a location bias. Therefore, we examined ages that were represented across all locations (i.e., 6-, 7-, and 8-year-olds) ($n = 54$; fourteen 6-, twenty-

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Table 1. Univariate results from the multivariate analysis of variance to test the effect of age on otolith elemental composition (n = 54; fourteen 6-, twenty-one 7-, and nineteen 8-year-olds).

Effect	MS	F	p
Mg:Ca			
Age	0.002	0.11	0.90
Error	0.02		
Mn:Ca			
Age	0.06	0.78	0.46
Error	0.08		
Zn:Ca			
Age	0.03	0.59	0.56
Error	0.04		
Sr:Ca			
Age	0.002	0.47	0.63
Error	0.005		
Ba:Ca			
Age	0.04	2.0	0.15
Error	0.02		
Pb:Ca			
Age	0.20	2.2	0.12
Error	0.09		

Note: All element-to-Ca ratios were log-transformed. Age degrees of freedom (df) = 2, error df = 51.

one 7-, and nineteen 8-year-olds). All standardized otolith element-to-Ca ratios were log-transformed to obtain normality and homogeneity of variance. There were no significant differences in the element-to-Ca ratios among the ages examined (multivariate F = 1.1, p = 0.35; Table 1). Consequently, all fish (n = 91) were included in subsequent analyses.

Otolith element-to-Ca ratios varied significantly among locations for all ratios examined except Pb:Ca (multivariate F = 6.9, $p < 0.001$; Table 2 and Fig. 3). A consistent pattern was observed for all element-to-Ca ratios except Sr:Ca. For the other five elements, the largest ratios were observed at Brookings and the smallest were observed at Gold Beach. The ratios at Grays Harbor and Cape Arago were intermediate and often similar with the exception of Mn:Ca, which was much lower at Cape Arago. For Sr:Ca, Grays Harbor had the largest ratios and the other three locations were similar.

DFA was used to predict collection location based on otolith elemental composition. Classification success was greatest using Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca, which all varied significantly among collection locations and were significant variables in the model ($F > 5.6$, $p < 0.01$). Average classification success was 73% with a jackknifed average of 64% (Table 3). The first two canonical discriminant functions explained 94% of the variation (Table 4).

Prior probabilities, based on random chance and sample size, predict that an average of 26% of the fish would be correctly classified. A classification average of 73% and a jackknifed average of 64%, which is probably more representative of the true discriminatory ability, is a significant improvement (Table 3). However, misclassifications were typically not to the nearest geographic neighbor but were spread relatively equally among locations.

Table 2. Univariate results from the multivariate analysis of variance to test the effect of collection location on otolith elemental composition.

Effect	MS	F	p
Mg:Ca			
Location	0.17	12.0	<0.001
Error	0.01		
Mn:Ca			
Location	0.93	16.3	<0.001
Error	0.06		
Zn:Ca			
Location	0.23	5.9	<0.001
Error	0.04		
Sr:Ca			
Location	0.03	5.7	<0.001
Error	0.005		
Ba:Ca			
Location	0.25	16.7	<0.01
Error	0.02		
Pb:Ca			
Location	0.23	2.5	0.07
Error	0.09		

Note: All element-to-Ca ratios were log-transformed. Location degrees of freedom (df) = 3, error df = 87.

Table 3. Classification success for black rockfish (*Sebastes melanops*) adults based on otolith microchemistry and microsatellite DNA.

	Classification success (% correct)		
Location (n_1, n_2)	Jackknife estimate	Five loci	Seven loci
Grays Harbor (22, 50)	59	59.7±2.1	68.9±1.0
Cape Arago (26, 33)	50	72.7±1.4	86.2±0.7
Gold Beach (26, 49)	85	43.5±1.2	68.4±2.1
Brookings (17, 17)	59	79.5±1.5	NA*
Average	64	63.1±0.6	74.6±0.6

Note: Sample size (n_1, n_2) for microchemistry (n_1) and microsatellites (n_2) are presented. For microsatellites, classification success was based on resampled allele frequencies (N = 1000). Ten iterations per population were generated and averaged for five and seven loci. The percent correct (± standard deviation) is included.

*Brookings was not included in the classification using all seven loci because *Spi*4 and *Spi*10 failed to meet the assumption of random mating at that location.

Table 4. Standardized coefficients for canonical discriminant functions (CDF) 1, 2, and 3 for the log-transformed element-to-Ca ratios used to classify black rockfish (*Sebastes melanops*) to collection location.

Element	CDF 1	CDF 2	CDF 3
Mg:Ca	0.61	0.48	0.03
Mn:Ca	0.35	–0.88	–0.47
Sr:Ca	0.40	–0.44	0.82
Ba:Ca	0.58	0.57	0.09

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Fig. 3. Mean otolith element-to-Ca ratios ($\times 10^3$) for black rockfish (*Sebastes melanops*) collected from four locations. Mean values ± standard errors are presented. GH, Grays Harbor (n = 22); CA, Cape Arago (n = 26); GB, Gold Beach (n = 26); and BR, Brookings (n = 17).

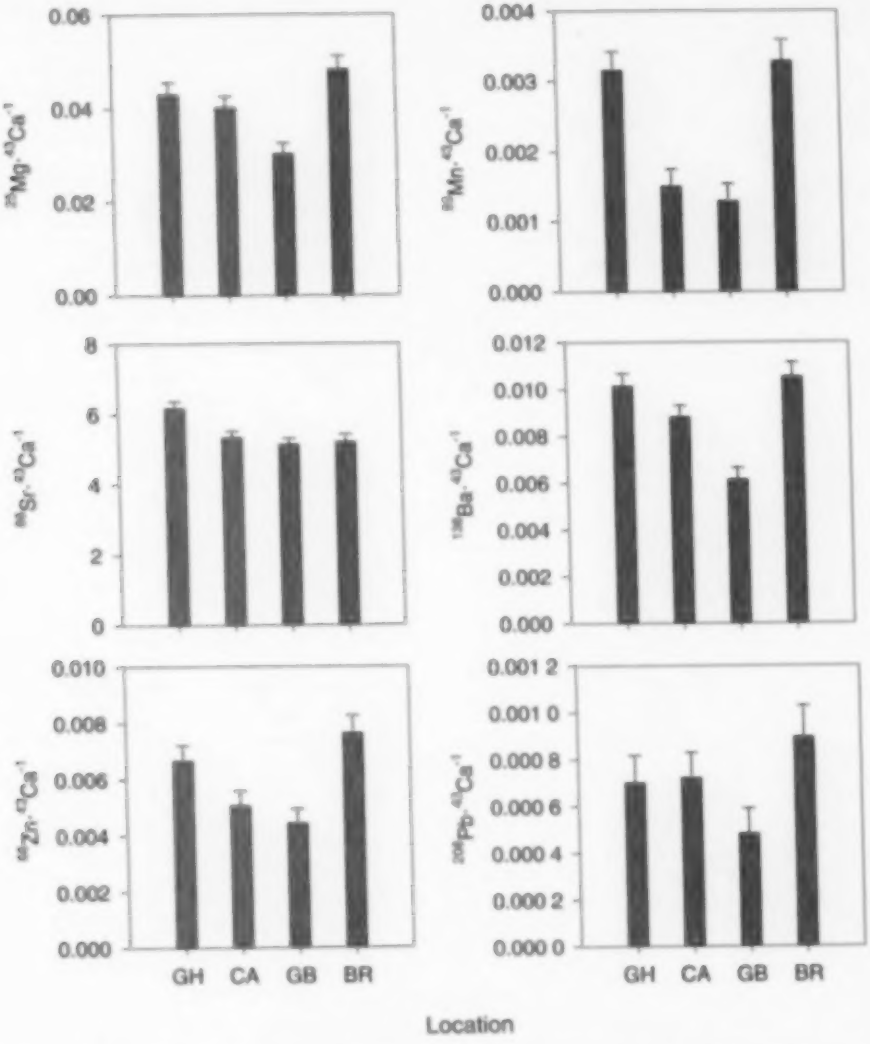

Table 5. The total (A_T) and mean (A_M) number of alleles per population, the observed (H_{obs}) and unbiased expected (H_{exp}) heterozygosities, and the probability of heterozygote deficiency (p).

Location	A_T	A_M	$H_{obs(7)}$	$H_{exp(7)}$	$p_{(7)}$	$H_{obs(5)}$	$H_{exp(5)}$	$p_{(5)}$	$F_{IS(7)}$	$F_{IS(5)}$
Grays Harbor	60	8.6	0.535	0.578	0.09	0.601	0.638	0.49	0.078	0.059
Cape Arago	47	6.7	0.484	0.485	0.49	0.542	0.546	0.41	0.003	0.009
Gold Beach	55	7.9	0.577	0.577	0.21	0.616	0.619	0.18	0.000	0.005
Brookings	41	5.9	0.512	0.557	0.003	0.631	0.637	0.30	NA*	0.010

Note: Heterozygosity and F_{IS} data are presented using both all seven and five loci (*Spi*6, *Spi*12, *Sal*3, *Sal*6, and *Sma*7) and are indicated by subscripts 7 and 5, respectively.

*$F_{IS(7)}$ was not calculated for Brookings because samples from that location failed to meet the assumption of random mating at *Spi*4 and *Spi*10.

Microsatellite DNA

The genetic statistics are summarized, calculated by collection location, in Table 5. Over all loci, the number of alleles ranged from 3 to 16 and the expected heterozygosities varied between 0.154 and 0.856. There was no evidence of gametic disequilibrium for any pair of loci within the samples. However, by location, deviations from HWE proportions occurred in the Brookings sample, which failed to meet the assumption of random mating at *Spi*4 and *Spi*10. We approached this problem in two ways. First, we com-

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Table 6. Significance tests for pairwise comparisons of F_{ST} in the black rockfish (*Sebastes melanops*).

Location	Grays Harbor	Cape Arago	Gold Beach	Brookings
Grays Harbor	—	0.032*	0.011*	0.021*
Cape Arago	—	—	0.015	0.026
Gold Beach	—	—	—	0.008

*Statistical significance at the adjusted nominal level (5%) for multiple comparisons, 0.0083.

pleted subsequent analyses with all four collection locations using only the five loci that did meet the assumption of random mating. Second, we completed the same analyses with all seven loci but without the Brookings sample, leaving three collection locations. We present results from analyses with all four locations and the five loci that met the assumption of random mating. Results from the analyses with three locations and seven loci are presented only when they differ.

F_{IS} values, or inbreeding coefficients, were comparable in both analyses, ranging from 0.00 to 0.078 (Table 5). Overall, F_{ST} (± standard error (SE)) = 0.018 ± 0.004 and was statistically greater than zero ($p < 0.001$). The exclusion of Brookings from the analysis did not significantly change the parameter value (F_{ST} = 0.017 ± 0.004). Pairwise comparisons of F_{ST} identified Grays Harbor, Washington, fish as significantly different from all Oregon samples (Table 6). There were no significant differences observed among Oregon samples. F_{ST} increased slightly when all Oregon samples were combined (F_{ST} = 0.02 ± 0.006). G_{ST} estimates were similar to F_{ST}.

Classification success by WHICHLOCI based on resampled populations (N = 1000) varied with the number of loci and among collection locations. The average success rate for all four collection locations and the five loci that met the assumption of random mating was 63.1% ± 0.6 SD. Classification success with only three collection locations (Brookings was removed) and all seven loci averaged 74.6 ± 0.6% SD (Table 3).

Discussion

Otolith microchemistry and microsatellite DNA data yielded corroborative and complementary information on population structure in black rockfish along the Oregon–Washington coast. We were able to classify 64% of the black rockfish to their collection location based on otolith microchemistry. Microsatellite data indicated significant genetic divergence among samples collected 340–460 km apart. Using five and seven loci, 63% and 75% of the fish were accurately assigned to collection locations, respectively. Thus, overall classification success was similar with both methods and significantly greater than that expected by chance alone (25%). These data indicate that there is population structure in black rockfish at scales that are <500 km and that otolith microchemistry and microsatellite DNA may prove useful in providing corroborative information on population structure in marine fishes.

Because we lack a thorough understanding of the mechanisms that regulate otolith elemental incorporation, there is concern that observed otolith composition may reflect differences in temperature, salinity, or growth rate rather than actual differences in the elemental composition of the water (Fowler et al. 1995; Gillanders 2002; Elsdon and Gillanders 2002). Most studies have found that salinity effects commonly occur over a salinity range of 10 to 30 practical salinity units (PSU) (Thresher 1999; Elsdon and Gillanders 2002). As all of the collection locations in this study are nearshore marine waters, where salinity is fairly constant and typically exceeds 30, it is unlikely that salinity effects occurred. If oceanographic factors, such as temperature, altered the incorporation of trace elements into otoliths, these factors would still represent differences in water characteristics among locations, and thereby support the premise that these fish were in different water masses. Clearly, a better understanding of the relationship between otolith elemental composition and water chemistry is needed.

There was no evidence of significant differences in size at age among these collection locations, which reduces the probability of growth-rate effects. Although we could not test the effect of age on otolith elemental composition across all ages examined, there were no significant effects of age observed in 6- to 8-year-olds. Power analysis showed that our tests had $\beta \geq 0.80$ to detect a 6% difference in mean otolith element-to-Ca ratios of the 6- to 8-year-olds. Therefore, we believe the data support the conclusion that there were no age effects on the otolith elemental composition of *S. melanops* used in this study.

Microsatellite data provided evidence of genetic divergence in *S. melanops* collected >340 km apart. Based on the microsatellite data, fish collected in Washington were separated from those collected in southern Oregon. The level of genetic divergence observed in this study (F_{ST} = 0.018 ± 0.004) over a range of 500 km is remarkably similar to previous reports within the genus *Sebastes*. For example, Withler et al. (2001) examined five microsatellites in Pacific ocean perch (*Sebastes alutus*) from 14 sites in British Columbia, reporting a mean pairwise F_{ST} = 0.015 ± 0.008 over a geographic range of 800 km. Buonaccorsi et al. (2002) examined six microsatellites in copper rockfish (*Sebastes caurinus*) along the west coast of North America and found significant population subdivision (F_{ST} = 0.007) among coastal samples collected <900 km apart. Roques et al. (2002) examined eight microsatellites of North Atlantic redfish (*Sebastes mentella*) across a 6000-km geographic range. Although they observed variation in the level of population subdivision across this range, there was significant population division between samples from Newfoundland and the Grand Banks (F_{ST} = 0.015) that were <200 km apart. Roques et al. (2002) noted that this level of genetic divergence is high for marine fish in such close geographic proximity with no apparent barriers to dispersal.

How much exchange occurs among *S. melanops* populations along the Oregon–Washington coast? Both otolith microchemistry and microsatellite data misclassified 35% of the individuals. There are a number of possible explanations for these misclassifications, including the following three: (*i*) we did not fully describe the otolith elemental or genetic variation within a location; (*ii*) the classification algorithms were inadequate to predict collection location accurately with the available data; or (*iii*) those fish had only recently (i.e., within the last 3 or 4 months) entered that location and

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thus had different otolith elemental compositions and (or) came from genetically distinct groups. The first and second possibilities are not mutually exclusive. The third option is plausible because the otolith transect integrated over the last year of life. However, additional study is needed to determine which of these explanations is correct. On the other hand, mark–recapture efforts provide corroborative evidence from a third sampling technique. Mathews and Barker (1983) recovered >60% of adult *S. melanops* within their release location. Interestingly, all three techniques estimate that ~65% of adult *S. melanops* remain in relatively discrete geographic locations.

A greater understanding of realized larval dispersal distances and adult movement patterns are needed for the successful management and conservation of marine fish populations. The level of exchange among populations that is theoretically adequate to maintain genetic diversity could vary when both fishing effort and highly variable levels of natural recruitment are considered. The replenishment of a depleted population depends on the size and proximity of adjacent populations, as well as on the proportion of individuals from those populations that disperse widely enough to contribute to the depleted population. Therefore the recovery rate of such a population, particularly in a species with sedentary adults, could be substantially slower with 70% vs. 30% self-recruitment. Techniques that can provide such information will be invaluable for establishment of harvest regulations and conservation strategies, such as the design and placement of marine protected areas.

In conclusion, we determined that there were alongshore differences in the otolith element-to-Ca ratios of adult *S. melanops*. Using those ratios, we accurately predicted an individual's collection location 64% of the time. We also observed significant genetic differences in individuals collected <500 km apart. Using five and seven loci, we accurately predicted an individual's collection location 63% and 75% of the time, respectively. In this study, otolith microchemistry and microsatellite DNA analyses yielded corroborative and complementary estimates on the level of exchange among *S. melanops* populations. The combined evidence presented here indicates a relatively high level of philopatry in *S. melanops*.

Acknowledgements

Funding was provided by an Oregon Marine Collaborative Fellowship (OR Sea Grant, ODFW, OR Trawl Commission, NMFS, and COMES) and a NOAA NERR Graduate Research Fellowship to JAM and the Cooperative Institute for Marine Studies (MAB). We thank Oregon State University's WM Keck Collaboratory for their assistance with LA–ICPMS and E. Backus for field assistance. Three anonymous reviewers provided valuable comments that significantly improved this manuscript.

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Spatial variability in rockfish (*Sebastes* spp.) recruitment events in the California Current System

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Abstract: A general assumption regarding spatial patterns of recruitment variability is that species with similar early life history characteristics tend to covary in reproductive success over scales of 500–1000 km. These assumptions are based on evaluation of recruitments from independent stocks, as few studies have assessed synchrony in recruitment within broadly dispersed stocks over finer spatial scales. We used data on age composition and landings to generate regional time series of recruitment deviations for three species of rockfish in the California Current System (*Sebastes goodei*, *Sebastes entomelas*, and *Sebastes flavidus*). We then used correlation analysis, principal components analysis, and other methods to evaluate the degree of synchrony among recruitment events in these regions. Results show that 51%–72% of the year-to-year variability in recruitment is shared coastwide within these species, while a lesser but significant fraction of the variability is associated with finer scale geographic features.

Résumé : En ce qui a trait à la structure spatiale de la variabilité du recrutement, on assume généralement que les espèces qui ont des caractéristiques semblables au début de leur cycle biologique ont tendance à avoir une covariance dans leur succès reproductif sur des échelles de 500–1000 km. Ces présuppositions sont basées sur des évaluations du recrutement à partir de stocks indépendants, puisque peu d'études ont évalué conjointement le synchronisme du recrutement chez des stocks à vaste répartition à des échelles spatiales plus fines. Nous avons utilisé des données de composition en âge et de débarquements pour générer des séries chronologiques régionales des déviations du recrutement chez trois espèces de sébastes habitant le courant de Californie (*Sebastes goodei*, *Sebastes entomelas* et *Sebastes flavidus*). Des analyses de corrélation, des analyses des composantes principales et d'autres méthodes nous ont servi à évaluer le degré de synchronisme ente les épisodes de recrutement dans ces régions. Les résultats indiquent que 51–72 % de la variabilité inter-annuelle dans le recrutement est commune à ces espèces sur toute la côte, alors qu'une fraction moins importante, mais significative de la variabilité est associée à des caractéristiques géographiques à échelle plus fine.

[Traduit par la Rédaction]

Introduction

Quantifying the physical and biological mechanisms responsible for spatial and temporal variability in recruitment remains a principal focus of fisheries biologists and oceanographers. To date, the majority of compelling hypotheses regarding density-independent processes are based on a consideration of physical forcing on both abiotic and biotic environmental conditions of early life history stages of marine fishes (Lasker 1978; Sinclair 1988; Bakun 1996). Houde (1989) demonstrated that as a result of declining mortality with increasing size for most larval fishes, the lower growth rates observed in cooler water species are usually associated with longer stage durations and higher cumulative mortality rates. Consequently, the importance of physical processes in determining year-class strength tends to be greater for species in the temperate and subarctic zones, where the influence of seasonality and variability in the physical and biological environment is potentially greater. This observation is echoed by Longhurst (2002), who found that longevity tends to be related to recruitment variability for many temperate and subarctic species of marine fish.

In general, the assumption has been that there tends to be synchrony in year-class strength (and marine survival for anadromous species) across fairly broad regions for populations with similar early life history characteristics and exposure to environmental variability. Botsford and Lawrence (2002) showed considerable spatial and temporal synchrony in coho salmon (*Oncorhynchus kisutch*) and Dungeness crab (*Cancer magister*) catches among ports and regions in the California Current System (CCS) between 1950 and 1990; however, they found that Chinook salmon (*Oncorhynchus tshawytscha*) landings did not covary over space and time. Similarly, Peterman et al. (1998) explored patterns of covariation in marine survival for sockeye salmon (*Oncorhynchus nerka*), and Pyper et al. (2001) examined the same for pink

Received 22 September 2004. Accepted 25 March 2005. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on 15 September 2005.
J18319

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 doi: 10.1139/F05-134 © 2005 NRC Canada

salmon (*Oncorhynchus gorbuscha*) throughout a large portion of their range in the northeast Pacific Ocean. Both studies showed moderate to strong covariation within regions, yet these patterns broke down among stocks separated by more than several hundred kilometres. Mueter et al. (2002) expanded on these studies by reevaluating correlations in spatial patterns of marine survival in sockeye and pink salmon and estimating the same for both chum salmon (*Oncorhynchus keta*) and the physical environment. They found that spatial correlations were greater for chum salmon than for other species, and tended to be fairly large for several important physical ocean properties. Upwelling indices had the strongest spatial correlation (on the order of 1000 km), with sea-surface temperature (SST) having intermediate spatial consistency (on the order of 400–800 km) and sea-surface salinity showing the weakest patterns (less than 400 km); importantly, these correlations tended to be stronger during the winter. Chelton (1981) and Chelton and Davis (1982) had similarly found that sea level, upwelling winds, ocean temperatures, and salinity tended to covary throughout much of the CCS, with subsurface temperatures and transport showing stronger spatial synchrony than surface temperatures and salinity.

Hollowed et al. (1987) and Hollowed and Wooster (1995) demonstrated synchrony of extreme year classes among a variety of stocks in different regions of the northeast Pacific Ocean, with stocks tending to be in phase within regions and out of phase between regions. Williams and Quinn (2000) also showed that recruitment in Pacific herring (*Clupea pallasii*) populations was correlated over fairly large spatial scales, although there was some separation between Alaskan and British Columbian stocks. In the North Atlantic Ocean, Koslow (1984) and Koslow et al. (1987) demonstrated spatial synchrony in year-class strengths of Atlantic groundfish stocks with similar early life history characteristics, and linked this variability to environmental forcing. Myers et al. (1997) further quantified this synchrony for a range of fishes, based on an exponential-decay model, and found that spatial scales of correlation in recruitment between marine fish stocks were typically on the order of 500 km. Finally, Schwartzlose et al. (1999) have shown basin-scale (>1000 km) synchrony in the recruitment of many coastal pelagic species in boundary current ecosystems, presumably linked to basin-scale or global climate forcing.

This study extends the analysis by the above authors by explicitly evaluating the degree of spatial synchrony in recruitment over finer geographic scales. To do this, we use port-specific catch-at-age data to generate regional, independent assessment models from which we derive time series of local recruitment deviations for chilipepper, widow, and yellowtail rockfish (*Sebastes goodei*, *Sebastes entomelas*, and *Sebastes flavidus*, respectively) in the CCS. These three species are reasonably data-rich, commercially important (they collectively represent over 55% of US landings of *Sebastes* spp. coastwide between 1981 and 2003), and are winter spawners that spend between 130 and 160 days in the pelagic environment following extrusion (Wyllie-Echeverria 1987; Woodbury and Ralston 1991). We evaluate the degree of geographic synchrony by several means, including the use of pairwise correlations between port groups, evaluation of shared variability with principal components analysis (PCA), and use of the exponential relationship between covariance and space as modeled by Myers et al. (1997). Interest in understanding temporal and spatial variability in recruitment and production has increased in recent years, fueled in part by a growing recognition of the importance of dispersal both in quantifying population dynamics and in considering the feasibility and design of spatially based management measures (Warner et al. 2000; Botsford et al. 2003).

It is not the intention in this work to draw inferences regarding stock structure or movement patterns. Widow and chilipepper rockfish have historically been managed as a single stock in the US portion of their range (Ralston et al. 1998; He et al. 2003), and widow rockfish in Canada are similarly treated as a single coastwide stock (Stanley and Haist 1997). Although Tagart (1991) found some evidence for stock differentiation in yellowtail rockfish, US stock assessments and management recommendations typically assume a coastwide stock stretching from Cape Mendocino to Vancouver Island, and Canadian assessments and management recommendations use that stock boundary along with a stock from Vancouver Island through the Queen Charlotte Islands (Stanley and Haist 1997). We have assumed that movement for all three species is minimal, which is consistent with what little is known of movement patterns for most *Sebastes* species. Stanley et al. (1994) report on 36 recoveries of yellowtail rockfish with recapture locations (from 14 000 tagged fish) from a Canadian tagging program off British Columbia and northern Washington, in which most moved less than 25 km before being recaptured, with a minority moving between 100 and 400 km. Pearcy (1992) found very little movement of adult yellowtail rockfish, based on acoustic tagging of 19 fish on Heceta Bank. Gunderson (1997) found that changes in the abundance and age composition of Pacific Ocean perch (*Sebastes alutus*) in response to fishing were highly localized, even decades after fisheries impacts, suggesting little to no migration of adults between areas of suitable habitat as close as 30 km. With regard to dispersal, we assume that pelagic larvae and juveniles are likely to be widely dispersed from their origin and that there is little latitudinal movement following settlement, such that the age structure of fish in different regions will tend to reflect the historical patterns of settlement by juveniles.

Materials and methods

Our general approach was to use regional landings and age-composition data to generate port- and region-specific age-structured models for chilipepper, widow, and yellowtail rockfish. References to port groups in the USA are consistent with the Pacific Coast Fisheries Information Network (PacFIN) hierarchical port-group structure, with the following exceptions: Eureka includes ports in the Crescent City and Brookings areas and Astoria includes both northern Oregon and all Washington ports in the Columbia International North Pacific Fisheries Commission area. Data on catch at age and landings were provided by the California Department of Fish and Game, the Oregon Department of Fish and Wildlife, the Washington Department of Fish and Wildlife, PacFIN, and the British Columbia Department of Fisheries

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Table 1. Number of subsamples and number of aged fish available by port group for chilipepper (*Sebastes goodei*), widow (*Sebastes entomelas*), and yellowtail (*Sebastes flavidus*) rockfish.

		Chilipepper rockfish		Widow rockfish		Yellowtail rockfish	
Port group	Latitude (°N)	No. of subsamples	No. of aged fish	No. of subsamples	No. of aged fish	No. of subsamples	No. of aged fish
Morro Bay (MRO)	35°20′	601	9 556	165	1 810	33	466
Monterey (MNT)	36°40′	922	13 309	517	4 935	129	1 136
San Francisco (SF)	37°50′	267	5 435	157	2 835	43	654
Bodega Bay (BDG)	38°20′	248	4 406	118	3 258	15	404
Fort Bragg (BRG)	39°30′	620	8 513	309	5 924	107	1 104
Eureka/CRS (ERK)	40°50′	372	3 013	732	16 640	352	5 737
Coos Bay (COOS)	43°20′			280	7 001	231	9 724
Newport (NEWP)	44°40′			730	18 869	360	12 949
Astoria (AST)	46°10′			801	34 810	855	59 561
Greys Harbor (US VN)	48°			313	19 120	579	33 833
Vancouver Island (CN VN)	49°			139	5 751	224	13 251
Queen Charlotte Islands (CHAR)	51°					251	16 544
Total		3030	44 232	4261	120 953	3179	155 363

Note: Boxes indicate port-group aggregations by species for each model.

and Oceans. Data-collection procedures for commercial fisheries for California, Oregon, and Washington are summarized in Sampson and Crone (1997) and for British Columbia in Stanley and Haist (1997). Where possible, annual-sample catch-at-age data were expanded to the total number of fish caught at age to arrive at the age composition of the catch. Where the associated catch data were not available, sampled fish were weighted equally in the age-composition data. As sampling generally occurred across a range of gear types, age data from different gears were pooled when cumulative catch-at-age curves showed no noticeable difference in selectivity. Where substantial differences in selectivity were evident, either gears were modeled as separate fisheries or data from minor gear types were excluded.

We developed data sets of catch at age and landings specific to port groups or regions from Point Conception, California, to the Queen Charlotte Islands off the British Columbia mainland. The number of sampled landings and aged fish available for each region are provided in Table 1. Boxes indicate the groupings of ports in aggregated models. For example, data for chilipepper rockfish were sufficient to have one model per port group between Morro Bay and Eureka, but there were not enough age data to model chilipepper rockfish north of Cape Blanco (the nominal boundary of the stock). By contrast, all ages and landings of yellowtail rockfish south of Cape Mendocino were included in one regional model, owing to the paucity of data for this region. The spatial extent of the study area, together with representative ports from most of the regions included in it, is shown in Fig. 1 (Fig. 1). Although the available data for this study included over 320 000 aged fish, for some early years only surface ages (i.e., whole otolith readings) were available. For example, most Oregon widow rockfish were aged with whole-otolith methods from 1979 through 1989 and by break-and-burn (Chilton and Beamish 1982) methods thereafter. Clark et al. (1987) found that surface age readings for yellowtail rockfish compared favorably with break-and-burn readings through age 15, but diverged significantly for older ages. To minimize any recruitment error associated with using surface ages for older fish, while maintaining as much consistency as possible with the coastwide assessments (which typically include up to 25 age classes), we modeled age groups only between the age of recruitment to the fishery (as estimated by the coastwide assessment) up to a maximum age of 20 years, with a plus group representing older fish.

Fig. 1. Sampling region, with representative ports associated with the models listed in Table 1.

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For building the regional models we used Coleraine, a generalized age-structured stock-assessment model developed by Hilborn et al. (2003). Coleraine is a spreadsheet template interfaced with AD Model Builder (Otter Research Ltd. 2000), in which parameter estimation is performed using maximum likelihood in the first step and a Bayesian approach in a second. This allows uncertainty around parameter estimates to be assessed from posteriors, although this generally was unnecessary for the purposes of this study. The model itself is a general structure for statistical age- and sex-structured models that allows for multiple fisheries, surveys, or other indices. Growth was modeled by the von Bertalanffy model, and as latitudinal gradients in size at age have been demonstrated for both widow and yellowtail rockfish (Pearson and Hightower 1991; Tagart 1991), estimates of the asymptotic length at age (L_∞), the Brody growth parameter (k), and the age at length 0 (t_0) were generated independently for each region. We found significant differences in size at age, with L_∞ increasing noticeably in more northerly regions, for all three species.

Recruitment in the model is estimated with a Beverton–Holt stock–recruit relationship:

$$(1) \qquad N^{1}_{t,a=1} = 0.5\frac{S_t}{\alpha + \beta S_t} e^{\left(\mathrm{Re}_t - \frac{\sigma_R^2}{2}\right)}$$

where is the number of age-1 fish at time $t + 1$ by sex (0.5 assumes a 50:50 sex ratio), α and β are the Beverton–Holt stock–recruit relationship parameters (derived from the parameters for the steepness of the spawner–recruit curve, h, and the recruitment at equilibrium in the absence of fishing, R_0). Re_t is the recruitment residual for year t (lognormally distributed with a mean of 0 and a prior on the standard deviation (σ) set by the user, we used a value of 1), and S_t is the spawning biomass in year t. The recruitment residuals, as they deviate from the expected recruitment, are the parameters of interest in this study because they represent the variations in year-class strength to be evaluated.

A realistic accounting of ageing error is critically important in estimating variability in year-class strength, especially when the majority of the landings are composed of older individuals and ageing error is significant (Tyler et al. 1989; Richards et al. 1997). Ageing-error matrices were generated directly from the most recent stock assessments for all three species by assuming a normal distribution of measured age for each true age based on the between-reader agreements reported in stock assessments. The percent agreement for chilipepper rockfish is estimated at 90% for age-1 fish, decreasing linearly to 30% agreement for age-21 fish (Ralston et al. 1998). Widow rockfish have 75% agreement for age-5 fish and 66% agreement for age-20 fish (He et al. 2003). Yellowtail rockfish are the most imprecise, with roughly 52% agreement for age-5 fish and just over 25% agreement for age-20 fish (Tagart et al. 2000). The predicted age composition is estimated by Coleraine in proportions of the catch by year, age, sex, and gear. For fitting to the observed age-composition data, Coleraine uses a robust likelihood formulation based on Fournier et al. (1998). The sample sizes used with the age-composition data (which scales the variance) were the number of subsamples (landings in which fish were sampled and aged) rather than the number of aged fish as in He et al. (2003) and Lai et al. (2003).

Estimates of maturity, sex-specific natural mortality, and length–weight relationships were taken directly from the most recent stock assessments as well. We did not, however, incorporate the increasing natural mortality rate for female yellowtail rockfish that has been modeled in past assessments. Although there is evidence to suggest that differences in natural mortality rate in older females of some species are significant (Leaman 1991; Tagart 1991), the use of sex-specific dome-shaped selectivity curves in assessment models should lead to similar results when they are fitted to catch curves (MacCall 1999). Moreover, different potential assumptions regarding varying natural mortality rates for older females would presumably be of greater consequence to estimates of stock status, not recruitment variability. Finally, we used the biomass estimates from the most recent coastwide assessments of each species as survey indices in each of the regional models (with the survey catchability coefficient set as a free parameter) in order to guide the biomass trajectories in the direction of those in the coastwide assessment. For these indices we assumed a coefficient of variation (CV) of 0.2 and used a range of sensitivity tests, such as replacing the trend from the coastwide assessment with a flat line, or inverting the trend entirely, to demonstrate that these indices were neither driving strong year classes nor dominating the trajectory of the model-estimated biomass.

A key objective throughout this effort was to be as consistent as possible with the most recent coastwide assessments. However, in evaluating the results, we use a range of model complexities in order to test the robustness of our results to differences in model structure. Coleraine uses nonlinear estimation procedures to fit the model to the available data in phases; consequently, we ran a suite of models with increasing complexity for each region and compared the results among models that were similar in structure. The first set of models included the estimation of the catchability coefficient, the equilibrium recruitment, the age at full selectivity (sex-specific), and the recruitment deviations by year. The second set of models included freeing up the left and right sides of the selectivity curve, implemented as a double half-Gaussian function of age (Hilborn et al. 2003), which generally improved the model fit significantly. The third set of models was the least parsimonious, wherein the parameters estimated included steepness, initial exploitation rates, natural mortality rate by sex, and error around the age at full selectivity. While implausible results were excluded, considerable latitude was granted to this set of models in order to evaluate what might be the best fitting model relative to model structure, and Akaike's Information Criterion was used as a measure of model improvement (Akaike 1992). In many cases, freeing up these additional parameters did not substantially improve model fit. Most importantly, the ultimate results varied little among these three sets of models. Consequently, we focus our discussion on the results from the second set of models (i.e., those with the left and right sides of the selectivity curve freely estimated).

We used both correlation methods and PCA to evaluate how much variability in the recruitment estimates within each species was shared coastwide. PCA has a long history

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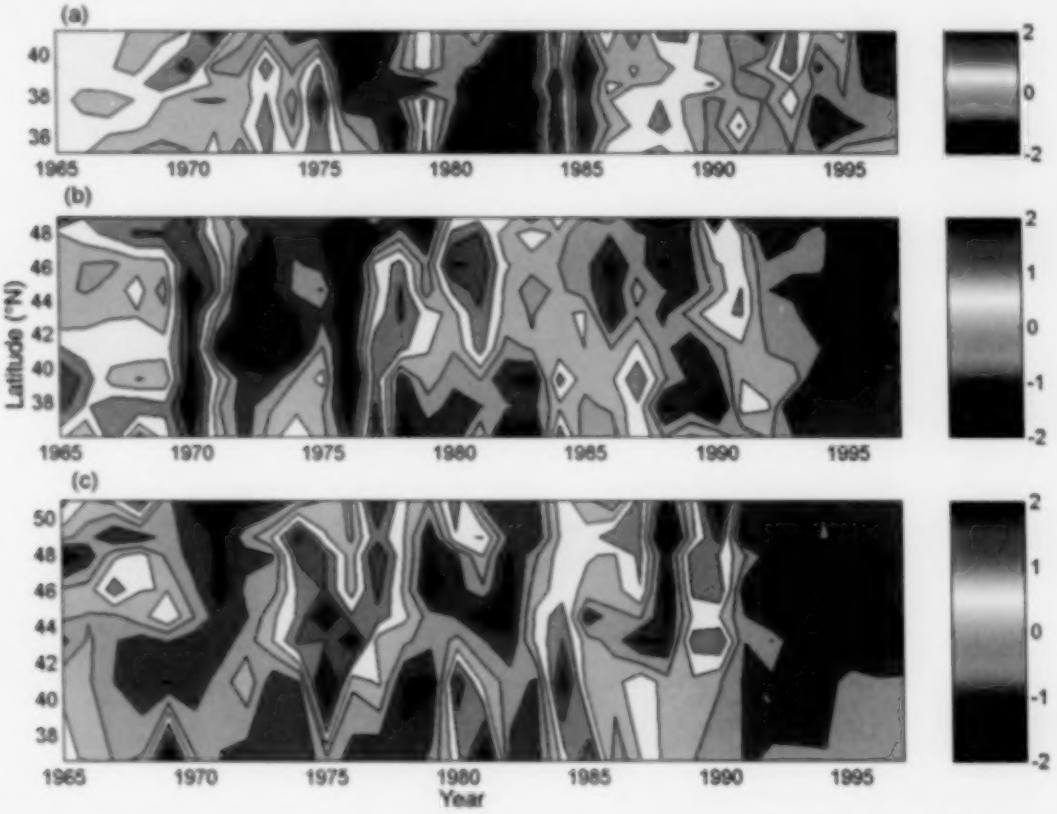
Fig. 2. Log recruitment deviations plotted over space and time for (*a*) chilipepper rockfish (*Sebastes goodei*), (*b*) widow rockfish (*Sebastes entomelas*), and (*c*) yellowtail rockfish (*Sebastes flavidus*) in the California Current System. Space corresponds to the latitude (*y* axis) of the ports or regional model as listed in Table 1.



of use in climatology, and is increasingly used in fisheries and oceanography to identify common modes of variability in data sets (Mantua et al. 1997; Hare and Mantua 2000; Botsford and Lawrence 2002). The method takes a covariance matrix consisting of spatially and temporally defined data and uses eigenanalysis to generate sets of principal components (PCs, or scores), in which each is an orthogonal linear combination of the original variables. The amount of variability in the raw data that each PC explains is a function of the eigenvectors (loadings) in which positive values represent positive correlations with the PC. The eigenvalues associated with each PC determine the fraction of the total variance explained by that particular PC. To estimate standard error and evaluate significance, we followed North et al. (1982), who showed that eigenvalues that did not overlap (when bounded by standard error) were likely to be significant, while those that did overlap were not. The modeled time periods were 1968 and 1997 for chilipepper rockfish and 1966 and 1995 for both widow and yellowtail rockfish, based on the availability and resolution of the age-composition data and the age of recruitment to the fishery. Pearson's correlation coefficients were simply estimated directly from raw time series for the time periods described above.

We calculated a third statistic to measure spatial synchrony in time series of recruitment, based on Myers et al. (1997). Those authors used available stock-assessment results for a wide range of species and assessed the spatial scale of recruitment variability by estimating the distance over which pairwise correlations between time series decayed with the equation

$$(2) \qquad \rho(d) = \rho_0 \, e^{-\frac{d}{v}}$$

where ρ_0 is the correlation between two stocks at zero separation, v is the exponential decay rate (or e-folding scale), and d is the distance between spawning locations of each population. The model is fit with nonlinear least squares and, because they found that it model was rarely improved by estimating ρ_0 independently, we evaluated this model both with ρ_0 free and with ρ_0 constrained to be 1.

Results

The recruitment time series from the regional catch-at-age models appeared to be fairly sensitive to sample sizes, with higher estimated CVs in recruitment time series for models with small sample sizes and lower CVs for models with larger sample sizes. The models with the highest recruitment CVs included chilipepper rockfish in Eureka (1.5), yellowtail rockfish south of Mendocino (1.4), yellowtail rockfish in the Eureka area (1.3), and widow rockfish in San Francisco –

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Table 2. Correlation coefficients between log recruitment deviations by port-group model as defined in Table 1.

(A) Chilipepper rockfish

	MNT	SF	BDG	BRG	ERK
MRO	0.83	0.77	0.60	0.59	0.34
MNT		0.88	0.74	0.72	0.41
SF			0.77	0.85	0.44
BDG				0.79	0.48
BRG					0.49

(B) Widow rockfish

	SF	BRG	ERK	COOS	NEWP	AST	US VN	CN VN
MNT	0.62	0.70	0.33	0.26	0.27	0.16	0.17	0.33
SF		0.71	0.57	0.35	0.43	0.30	0.37	0.43
BRG			0.61	0.51	0.51	0.36	0.23	0.32
ERK				0.76	0.69	0.63	0.53	0.45
COOS					0.83	0.76	0.56	0.49
NEWP						0.90	0.63	0.51
AST							0.70	0.53
US VN								0.75

(C) Yellowtail rockfish

	ERK	COOS	NEWP	AST	US VN	CN VN	CHAR
MNT	0.15	–0.15	–0.14	0.08	0.06	0.08	0.05
ERK		0.38	0.40	0.35	0.40	0.39	0.31
COOS			0.76	0.58	0.58	0.52	0.30
NEWP				0.75	0.70	0.41	0.40
AST					0.91	0.64	0.65
US VN						0.69	0.62
CN VN							0.49

Note: Port groups are arranged south to north. A white font on black denotes a p value less than 0.01, a white font on dark grey denotes a p value between 0.01 and 0.05, and a black font on light grey denotes a p value between 0.05 and 0.10.

Bodega Bay (1.2), all of which had sample sizes between 3000 and 5000 aged fish. By contrast, recruitment time series for regions with over 10 000 aged fish typically had CVs between 0.6 and 1.0. Whether such differences in variability resulted from differences in sample size or were a consequence of some other mechanism altogether is unclear; however, we made no effort to correct for this effect. The average CV of all of the port-specific models was 0.9, which was not appreciably higher than the CVs from the most recent assessments of 0.8, 0.7, and 0.6 for chilipepper, widow and yellowtail rockfish, respectively.

For the sake of brevity, we do not report all of the resulting recruitment estimates by species, area, and model. Instead, we present the results graphically (Fig. 2) as standardized anomalies of the log-transformed recruitments plotted as contours over space and time, and one can appreciate visually the nature of spatial synchrony in recruitment anomalies by species. For example, chilipepper rockfish recruitment was strong coastwide in 1973, 1975, 1984, and 1989. Weak years were apparent in 1976–1977, 1980–1983, and 1993–1997. Recruitment of widow rockfish was very strong along their entire latitudinal range in 1970 and most of their range in 1977, with poor recruitment between 1972 and 1976 and between 1993 and 1997. There appeared to be more spatial variability in several years, with the 1980 year class stronger north of 42°N and the 1984 and 1987 year classes stronger south of 42°N (Cape Blanco). Yellowtail rockfish exhibited the greatest spatial distribution of the three species, and showed synchrony throughout most of their range in several years, such as 1976–1978, 1984–1985, and 1989. Weak recruitment is suggested for the early 1970s, 1983, and nearly all of the 1990s. It is possible that ageing error contributed to some of the slight offsets in strong year classes between regions. However, there is clearly a pattern of synchrony in year-class deviations for this population, with either more error or more spatial variation than in the other two species.

We show all possible pairwise correlation coefficients for the time series of the logarithm of annual recruitment estimates by port group (Table 2). These results also demonstrate a high degree of correlation among most "stocks" by species, particularly between adjacent port groups. The most notable exception is yellowtail rockfish for the Monterey region, which includes all catch and age data collected between Morro Bay and Fort Bragg. Recruitment in this region was essentially uncorrelated with that in all other yellowtail rockfish regions. The weakest correlations in both widow and chilipepper rockfish regions also seem to be associated with differences between the areas north and south of Cape Mendocino.

The results of the PCA also demonstrate a high degree of spatial synchrony between the ports and regions modeled. The first PCs explained approximately 72%, 57%, and 51% of the variability in recruitment over time for chilipepper, widow, and yellowtail rockfish, respectively. Loadings were quite even across the spatial variables (Fig. 3), and were

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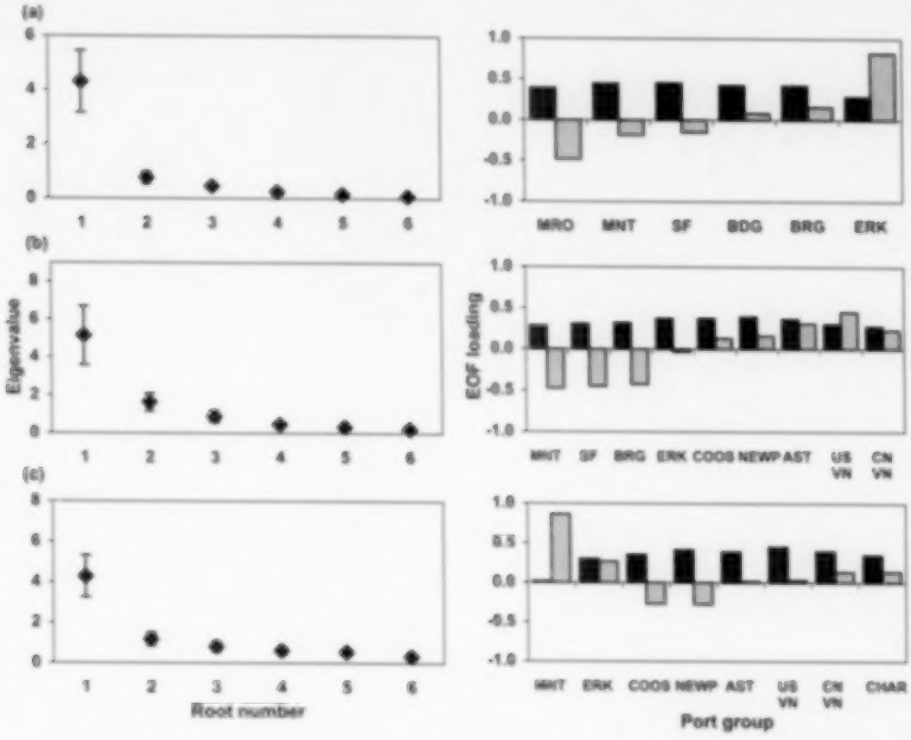
Fig. 3. The first six eigenvalues (left-hand panels) and loadings of the first (solid bars) and second (shaded bars) empirical orthogonal functions (EOFs) over port groups (right-hand panels) for (*a*) chilipepper, (*b*) widow, and (*c*) yellowtail rockfish. Port groups are as follows: Morro Bay (MRO), Monterey (MNT), San Francisco (SF), Bodega Bay (BDG), Fort Bragg (BRG), Eureka and Crescent City (ERK), Coos Bay (COOS), Newport (NEW), Astoria (AST), Greys Harbor (US VN), Vancouver Island (CN VN), and Queen Charlotte Islands (CHAR).



highly significant for all three species. Interestingly, the loadings on the second PC for both chilipepper and widow rockfish seem to explain major regional differences in year-class strength. The signs of the loadings on these PCs reverse from south to north, with the greatest differences again occurring between stocks separated by Cape Mendocino (Fig. 3). This was especially apparent in yellowtail rockfish, where the Monterey stock had virtually no relationship to the first PC but had the greatest loading on the second. Interestingly, if the Monterey time series is excluded from the PCA for yellowtail rockfish, the first PC explains 61% of the variability, with even loadings among locations. The first two PCs, or scores, for each of the three species over time are also shown (Fig. 4).

We tested the sensitivity of the results both to changes in model structure and to the nature of the data used in the PCA (normalized log recruitments, residuals from the spawner–recruit curve, or linearly detrended recruitments). We found that the results changed very little with regard to the analysis when accounting for any of these potential factors. For example, the eigenvalues, loadings, and PCs presented here were based on the logarithm of the resulting recruitments from the suite of models in which the left and right sides of the selectivity curves were freely fit. When only the age at full selectivity was free (simple models) the first PCs explained 73%, 57%, and 49% of the variability for chilipepper, widow, and yellowtail rockfish, respectively. When all parameters that improved the negative log likelihood (while meeting Akaike's Information Criterion) were free, the first PC of the resulting recruitments explained 72%, 52%, and 53% of the variability, respectively. When the effects of spawning-stock biomass were explicitly removed, by using the recruitment residuals rather than the estimated recruitments, the first PC explained 70%, 55%, and 51% of the variability, respectively. Similarly, when detrended time series of recruitments were used, the first PC explained 72%, 54%, and 51% of the variance, respectively. Finally, we obtained very similar results using both longer (35 years) and truncated (20 years) time series for each of the three species. In all instances, the trajectories of the PCs, or scores, and the loadings on the spatial variables changed very little.

We also evaluated our results with the exponential-decay model with ρ_0 both free and fixed at 1 (Fig. 5). With ρ_0 free, the estimate of ρ_0 was 0.90, and the e-folding scale, v, for all three species was estimated at 800 km, somewhat greater than the average of 500 km for all of the marine stocks estimated by Myers et al. (1997). With ρ_0 constrained to be 1, the estimate of v was 670 km. Although model fit was improved by freeing ρ_0, the improvement was modest, and was not significant based on Akaike's Information Criteria when likelihoods were used to fit the model. If we fit species-

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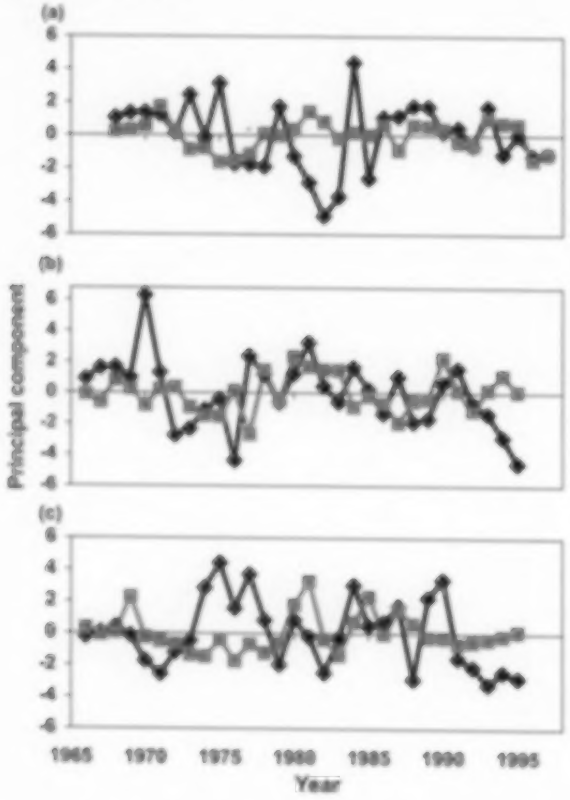
Fig. 4. Time series of loading values for first (solid symbols and lines) and second (shaded symbols and lines) principal components of recruitment for (*a*) chilipepper, (*b*) widow, and (*c*) yellowtail rockfish.



specific curves, the estimated v values were 720 (710), 990 (820), and 620 (550) km with ρ_0 free (fixed) for chilipepper, widow, and yellowtail rockfish, respectively. Myers et al. (1997) had previously estimated e-folding rates for North Atlantic *Sebastes* species as well as Pacific Ocean perch, a species for which Gunderson (1977) had earlier noted apparent synchrony over large spatial scales. They estimated a v value of 930 km for Pacific Ocean perch and 670 km for North Atlantic *Sebastes* species (both estimates made with ρ_0 fixed at 1), which suggested that *Sebastes* spp. stocks tended to show more synchrony than the overall average. The fact that our results are very similar to those of Myers et al. (1997) for *Sebastes* species in particular reinforces the notion that recruitment within this genus tends to be largely synchronous over broad spatial scales.

Discussion

Our results clearly demonstrate that there is substantial spatial synchrony in year-class strength over scales on the order of 500–1000 km for these, and possibly other, winter-spawning *Sebastes* spp. in the CCS. Intuitively, much of the spatial variability in year-class strength that does exist is associated with major geographic features such as Cape Mendocino and Cape Blanco, both of which have long been characterized as important physical and biological transitional zones in the CCS (Parrish et al. 1981; Connolly et al. 2001). Cape Mendocino would appear to be a more significant feature based on these results, although the limitations of the data are such that the actual location of catches is somewhat uncertain. Williams and Ralston (2002) also found that Cape Mendocino (and the Mendocino Escarpment) was one of the most noteworthy barriers to the latitudinal distribution of rockfish species.

These observations suggest that large-scale physical forcing mechanisms (1000s of kilometres) may be more important than regional (10s of kilometres) or mesoscale (100s of kilometres) processes in controlling recruitment (at least for the winter-spawning *Sebastes* spp. and at the spatial scales resolvable by this study), despite notable and important spatial differences between north and south of Cape Mendocino. Moreover, these results are consistent with other estimates of spatial scales of recruitment variability by Hollowed and Wooster (1995), as well as variability in landings of Dungeness crab and coho salmon estimated by Botsford and Lawrence (2002). Hannah (1995) also suggested that ocean shrimp (*Pandalus jordani*) recruitment events seem to be synchronous throughout the northern part of the CCS, and linked recruitment variability to large-scale physical forcing. Genetic evidence also suggests high homogeneity for many shelf rockfish species in the CCS. Hedgecock (1994) found that fish and invertebrates with planktonic larvae generally maintain low spatial genetic variance over large (500–2000 km) regions in the CCS, and analysis of a range of *Sebastes* species also suggests little genetic differentiation within the CCS region (Wishard et al. 1980; McGauley and Mulligan 1995; Rocha-Olivares and Vetter 1999).

One unresolved issue in this investigation is the apparent lack of synchrony in year-class strength among these three populations. Although occasional stronger or weaker year classes are shared among the three species, there are no significant correlations among the first PCs for any of the three species, nor among the recruitment deviations for these three stocks, based on the most recent coastwide stock assessments. However, there are strong correlations among these and other stocks, and a PCA of recruitment deviations among seven of the major coastwide stocks (based on stock-assessment results) suggests that some 33%–37% of the variance is shared in the first PC, with even loading across species (J. Field, unpublished data). Similarly, covariance among species is high in 21 years of midwater trawl estimates of pelagic juvenile rockfish abundance, with the 10 most abundant species sharing on the order of 80% of their variance over the 21-year time series (S. Ralston, unpublished data). Different exploitation histories may account for part of these discrepancies, since most of the recent estimates suggest that these stocks range from less than 10% to over 50% of their unexploited biomass, as might a wide range of other physical and biological factors.

Year-class strength in winter-spawning rockfish is thought to be set prior to the late pelagic juvenile stage, because there is strong coherence between the abundance of pelagic juveniles, estimated by midwater trawl surveys, and the abundance of settled (benthic) juveniles over spatial scales of 50–350 km (Ralston and Howard 1995). The abundance

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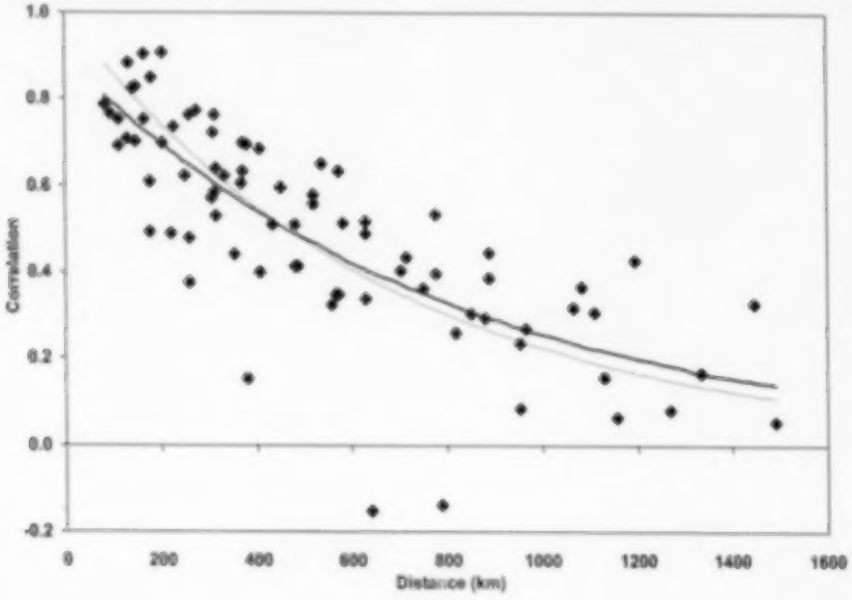
Fig. 5. Predicted correlation values across for the exponential-decay model with ρ_0 (the correlation at a distance of 0) both free (solid line) and fixed at 1 (shaded line). Points are the observed correlations between pairs of regional recruitment time series plotted against the distance between regions.



of pelagic juveniles also appears to be strongly correlated with the magnitude of winter (December–February) southward transport in the CCS (S. Ralston, unpublished data). This in turn also seems to have a strong pattern of spatial coherence, which is also reflected by the observation that coastal sea levels vary in phase along the entire west coast (Chelton and Davis 1982; Parrish et al. 2000). If year-class strength is set in the winter (post-extrusion) period, the large spatial covariance in year-class strength would also be consistent with the greater coherence in the physical ocean conditions (wind-derived upwelling, SST, and salinity) described by Mueter et al. (2002) for the winter (as opposed to spring and summer) seasons. By contrast, the salmon survival covariance patterns described by Mueter et al. (2002) and others (Peterman et al. 1998; Pyper et al. 2001) were on smaller spatial scales, perhaps because juvenile salmon survival primarily reflects ocean conditions in spring and summer, when moderate correlations at short distances declined much more rapidly over space than in winter.

An abundance of research has linked the productivity of commercially and ecologically important species throughout the northeast Pacific Ocean with large-scale climate variability, particularly interannual (El Niño/Southern Oscillation scale) and interdecadal (Pacific Decadal Oscillation (PDO) scale) climate forcing (Chelton et al. 1982; MacCall 1996; Francis et al. 1998). The PDO index, which is the leading PC of monthly North Pacific SSTs above 20°N, tracks the dominant patterns of SST variability in the North Pacific Ocean (Mantua et al. 1997). The PDO is closely associated with variations in the Aleutian Low pressure cell, which itself is the leading pattern of atmospheric variability in the North Pacific Ocean (Trenberth and Hurrell 1994). During positive phases of the PDO (which include the period between 1977 and 1998), coastal SSTs in both the Gulf of Alaska and the CCS tend to be higher, while those in the North Pacific Gyre tend to be lower; the converse is true in negative phases. Although this was first characterized with regard to changes in the productivity of salmon stocks, subsequent studies have identified links with many other marine populations, potentially including many of the west-coast rockfish that seem to have experienced poor reproductive success throughout the 1980s and 1990s (Hare and Mantua 2000; Sydeman et al. 2001; S. Ralston, unpublished data). From 1999 to 2002, ocean conditions seem to have improved for many populations, in association with lower coastal ocean temperatures, high rates of equatorward transport, and enhanced productivity of zooplankton and forage fish throughout the CCS. Such changes have been described as indicative of a possible 1999 phase shift in the PDO (Brodeur et al. 2003; Peterson and Schwing 2003). However, while the spatial patterns of warming and cooling over the last 4 years have been similar to those observed prior to 1977 for the CCS and other areas, they have been extremely different in others (Bond et al. 2003), and as the PDO index was mostly positive from 2003 through 2004, there are substantial uncertainties regarding future climate trends.

Juvenile-recruitment indices from the midwater trawl surveys have related well to recruitments estimated from coastwide stock assessments for several species, including chilipepper rockfish (Ralston et al. 1998), widow rockfish (Williams et al. 2000), and Pacific hake, *Merluccius productus* (Sakuma and Ralston 1997; Helser et al. 2002). Consequently, these recruitment indices have been incorporated into assessments as an index of year-class strength for these species. As Adams and Howard (1996) document density-dependent variability in natural mortality rates of newly

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settled juvenile blue rockfish (*Sebastes mystinus*), such that greater juvenile abundance is associated with a higher mortality rate, these indices are transformed prior to inclusion in the assessments. Given that recent surveys of juveniles cover a relatively modest (approximately 200 km) section of coastline, the evidence of spatial synchrony presented here supports the contention that these surveys are representative of larger scale trends in recruitment. The results may also have implications regarding larval dispersal and population connectivity, which might be particularly relevant to future spatially based management efforts. While marine protected areas and other spatial management measures are widely acknowledged to be an effective tool for protecting habitat and biodiversity, there remain questions regarding their impacts on future yield, many of which are centered on unanswered questions regarding the degree of connectedness and dispersal within populations (Warner et al. 2000; Hilborn et al. 2004). For example, Botsford et al. (2003) demonstrate that species with greater dispersal will likely require larger fractions of coastline in reserves if reserves are to function comparably to conventional fisheries management. While the results reported in this paper, taken alone, are not evidence of high rates of dispersal of rockfish larvae and juveniles, they do demonstrate that one reasonable expectation for high dispersal rates, synchrony in year-class strength over large spatial scales, is met for these (and presumably other) winter-spawning species.

It is increasingly evident that the low productivity, extreme longevity, and highly variable recruitment that characterize most rockfish populations are crucial life-history adaptations to their highly dynamic environment, adaptations that concurrently make such species vulnerable to overexploitation (Leaman and Beamish 1984; Parker et al. 2000; Longhurst 2002). The ability to directly and indirectly estimate year-class strengths from surveys and other sources, as well as to better understand the relationship between environmental conditions in the CCS, should improve short-term forecasts of productivity, biomass levels, and allowable catches from stock assessments. This in turn should lead to better long-term understanding and prediction of both production trends and rebuilding trajectories for overfished and depleted species.

Acknowledgements

This paper could not have been written without the cooperation of and the ageing data provided by Mark Freeman and Sarah Kennedy (Oregon Department of Fish and Wildlife), Jack Tagart and Cathy Evans (Washington Department of Fish and Wildlife), and Rick Stanley and Kate Rutherford (Department of Fisheries and Oceans Canada), as well as the many port samplers and ageing technicians coastwide who made such data available. Additional thanks are extended to Don Pearson for assistance with the California Cooperative Survey (CalCOM) database, William Daspit for PacFIN catch data, Han Lin Lai and Xi He for their assessment data and results, Billy Ernst for help using Coleraine, and Aditya Agrawal for help with Fig. 1. We thank Eric Bjorkstedt, Isaac Kaplan, Xi He, Alec MacCall, Don Pearson, and Brian Wells for their comments and suggestions on earlier drafts of the manuscript and two anonymous reviewers for their thorough and constructive comments. Funding was provided by the NOAA Fisheries Ecological Indicators Research Initiative.

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RNA/DNA ratio and total length of yellow perch (*Perca flavescens*) in managed and natural wetlands of a large fluvial lake

D. Tardif, H. Glémet, P. Brodeur, and M. Mingelbier

Abstract: RNA/DNA ratios and total lengths were compared to determine growth patterns of age-0 yellow perch (*Perca flavescens*) in managed and natural habitats of a large fluvial lake (Lake Saint-Pierre, St. Lawrence River, Quebec, Canada) over seasonal and yearly temporal scales. In 2002, the RNA/DNA ratio responded to degree-days accumulated over periods of 7–8 days before sampling, while in 2003, no relationship with temperature was established. The growth patterns obtained each year probably reflect indices responding to different limiting variables. In 2002, temperature would have been limiting, whereas in 2003, other factors such as prey availability, food quality, and competition may have influenced growth. In addition, the discrepancy between total length and RNA/DNA ratio observed in 2003 may reflect a differential time of response to limiting variables. These results together show that the two indices reflect growth at different time scales and suggest that their combination can help identify shifts between limiting environmental variables. Also, growth in managed wetlands during springtime was systematically superior to that in the natural environment, supporting the contention that managed wetlands are highly productive habitats. In natural habitats, growth rates were higher on the south shore by summer, which is consistent with the established north–south productivity gradient in Lake Saint-Pierre.

Résumé : La comparaison des rapports ARN/ADN et des longueurs totales chez des perchaudes (*Perca flavescens*) d'âge 0 nous a servi à déterminer les patrons de croissance dans des habitats aménagés et naturels d'un grand lac fluvial sur des échelles temporelles saisonnières et annuelles. Le rapport ARN/ADN en 2002 réagit aux degrés-jours accumulés sur des périodes de 7–8 jours avant l'échantillonnage, alors qu'en 2003 il n'y a pas de relation avec la température. Les patrons de croissance obtenus chaque année reflètent probablement des indices qui réagissent à différentes variables limitantes. En 2002, la température aurait été le facteur limitant, alors qu'en 2003 d'autres facteurs, tels que la disponibilité des proies, la qualité de la nourriture et la compétition auraient pu influencer la croissance. De plus, le désaccord entre les longueurs totales et les rapports ARN/ADN observé en 2003 peut peut-être s'expliquer par des temps différents de réaction aux variables limitantes. Dans leur ensemble, ces résultats montrent que les deux indices illustrent la croissance à deux échelles temporelles différentes; leur combinaison peut vraisemblablement aider à identifier les changements de variables environnementales limitantes. De plus, la croissance dans les terres humides aménagées au printemps est systématiquement plus forte que dans les milieux naturels, ce qui appuie l'idée que les terres humides aménagées sont des habitats de très grande productivité. Dans les habitats naturels, la croissance est plus forte en été sur la rive sud, ce qui est compatible avec le gradient nord–sud de productivité établi au lac Saint-Pierre.

[Traduit par la Rédaction]

Introduction

The establishment of measures that can determine growth rates and be informative about the influence of the environment during the first growing season is paramount for predicting recruitment and understanding the early life history of larval fish. Analyses of macromolecules, specifically nucleic acids, as a proxy for growth rate in fish are becoming increasingly popular (McLaughlin et al. 1995; McNamara et al. 1999; Weber et al. 2003). RNA/DNA ratios have been used to accurately estimate growth rate and feeding condition of larval marine fish and have been validated in laboratory and field conditions (Buckley and Lough 1987). Their use is based on the premise that while the quantity of cellu-

Received 22 October 2004. Accepted 11 April 2005. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on 21 September 2005.
J18375

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 doi: 10.1139/F05-137 © 2005 NRC Canada

lar DNA remains relatively unchanged, the quantity of RNA varies according to protein synthesis and thus reflects recent growth. Generally, well-fed and fast-growing larvae have a higher RNA/DNA ratio than slow-growing ones (Clemmesen 1994). The RNA/DNA ratio is considered an instantaneous measure of growth rate in the field (Buckley 1984) because it responds to changes in feeding conditions and growth after periods as short as 1–3 days in a variety of marine species. Conversely, total length is often used to infer somatic growth, and is considered as a long-term indicator of cumulative growth. Generally, total length reflects growth in response to the sum of all environmental influences experienced during a lifetime, and unless measured frequently, gives little information on short-term growth dynamics. Few studies have dealt with RNA/DNA ratios in freshwater fish, and have generally been restricted to laboratory-reared salmonid species (Ferguson and Danzmann 1990; Mathers et al. 1993; Malzahn et al. 2003). While these studies have focused on 1+ or older fish, to our knowledge not many have evaluated young-of-the-year (YOY) growth using RNA/DNA ratios combined with total length in a freshwater field setting.

The yellow perch (*Perca flavescens*) is of appreciable economical importance in North America, particularly in the Great Lakes – St. Lawrence River basin, owing to interest in its commercial and recreational uses. This species shows high growth-rate variation during the first year because of such factors as temperature (Staggs and Otis 1996), prey availability (Graeb et al. 2004), maternal effects (Heyer et al. 2001), and ontogenetic-niche shift (Whiteside et al. 1985) as well as intraspecific competition (Post and McQueen 1994). Only a few studies have used RNA/DNA ratios to evaluate percid growth in the context of either interspecific competition (Fullerton et al. 2000) or environmental metal contamination (Audet and Couture 2003). These studies have not directly addressed growth of yellow perch during their first year.

Lake Saint-Pierre, which is the largest fluvial lake (approximately 400 km^2) of the St. Lawrence River ecosystem, is an attractive study site because it exhibits a wide range of abiotic conditions. In this lake, yellow perch dominates the fish community, which is composed of up to 50 species (La Violette et al. 2003). The north and the south shores of the lake are shallow (<3 m) and separated by a deep (>10 m) navigation channel. Distinct water masses flowing along the two shores vary according to their spectral characteristics and transparency, and generate directly (i.e., depending on light penetration, nutrient and sediment concentrations) or indirectly (i.e., depending on vegetation type and density, water flow, substrate type) very diversified fish habitats and productivity gradients (Frenette et al. 2003). In addition to the contrast between the north and south shores, the floodplain of Lake Saint-Pierre includes a substantial surface area, approximately 1300 ha, which has been managed since the 1980s to compensate for fish habitat losses. The managed wetlands are surrounded by dikes and partially controlled to maintain stable water levels and accelerate water warming in the spring. Previous studies indicate that fish, particularly yellow perch, which is one of the most abundant species, use managed wetlands as spawning, nursery, and foraging habitats (Brodeur et al. 2004). The habitat gradients found in the Lake Saint-Pierre area represent an interesting opportunity to compare several growth indices measured in YOY yellow perch from managed wetlands and natural habitats.

In the present study we compare biochemical (RNA/DNA ratio) and classical (length) growth indices measured in YOY fish in response to a wide range of abiotic conditions found in the field. We defined the following three main types of habitats: (1) managed habitats and (2) natural habitats on the south shore and (3) natural habitats on the north shore of Lake Saint-Pierre. Growth of age-0 yellow perch was measured in each type of habitat during two time periods (spring and summer) and 2 consecutive years (2002 and 2003). We also explored the time scale over which the two indices respond to changes in temperature. We investigated RNA/DNA ratios during 12 short periods before sampling (1–12 days) and a long period from hatch to sampling, and total length during periods from hatch until spring and (or) summer sampling. A joint assessment of biochemical and classical indices was used to evaluate the ecological implications of differences in growth of yellow perch between managed wetlands and natural habitats.

Material and methods

Study area

This field study was undertaken at Lake Saint-Pierre, St. Lawrence River, Quebec, Canada (Fig. 1). Lake Saint-Pierre is a typical fluvial lake, classified as an ecological biosphere reserve by the United Nations Educational, Scientific and Cultural Organization and protected by the Ramsar Convention. At mean discharge, the flooded surface area is 31 500 ha, with a low mean depth of 3.17 m, forming a floodplain that reaches approximately 14 000 ha for 5–9 weeks, with a 2-year recurring flood event (Frenette et al. 2003). By springtime, fish can access the managed wetlands in the floodplain to spawn. After the spring flood, contact between the managed wetlands and the surrounding water masses is interrupted, and for several weeks, fish remain captive in the managed wetlands. Annual drawdown operations in late June and in October evacuate fish before ice cover to prevent high predation rates and winterkill (Lepage and Lalumière 2003). Yellow perch are subject to sport and commercial exploitation at the natural sites of Lake Saint-Pierre but not in the managed wetlands.

Natural study sites on the north shore include Maskinongé Bay (46°12′N, 72°58′W) and Yamachiche Bay (46°16′N, 72°50′W), while south-shore sites are represented by Longue Pointe (46°11′N, 72°45′W) and Anse du Fort (46°08′N, 72°53′W) (Fig. 1). Managed wetlands include Saint-Eugène (46°17′N, 72°39′W) and Saint-Barthélemy (46°10′N, 73°00′W) on the north shore and Île du Moine (45°11′N, 73°00′W) on the south shore.

Field sampling and laboratory analysis

Sampling of YOY yellow perch in managed wetlands and natural habitats was conducted over 2 years in the spring (28–31 May 2002, 30 May – 1 June 2003) and summer (18–21 June 2002; 21 June – 4 July 2003). During the spring period, fish were sampled using push nets (a 2 m long

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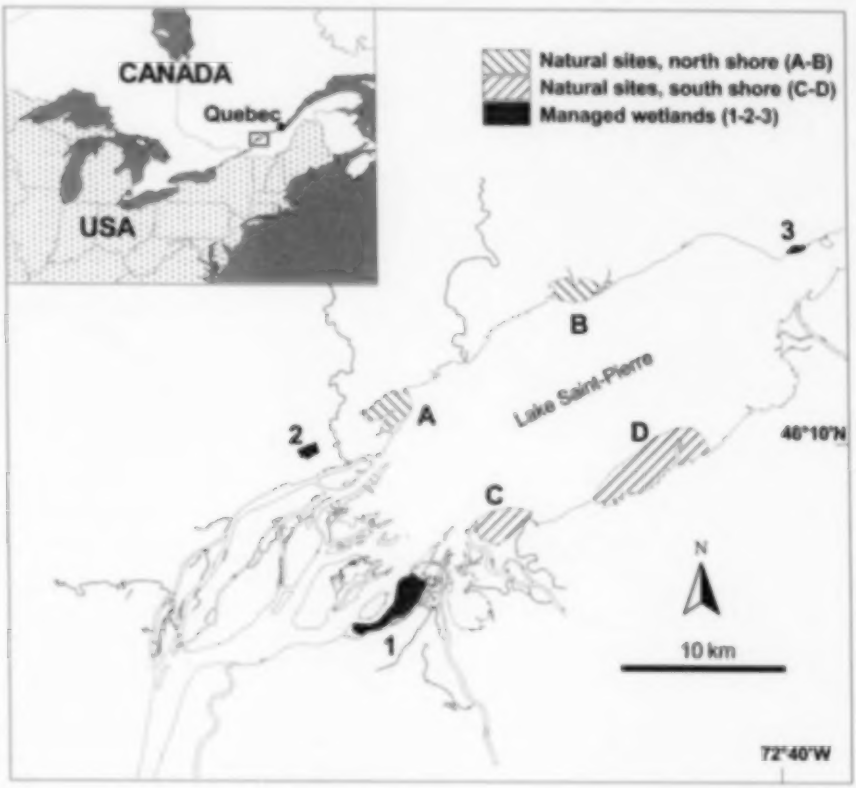
Fig. 1. Locations of managed wetlands (solid areas) and natural study sites (hatched areas) along the north and south shores of Lake Saint-Pierre, St. Lawrence River, Quebec, Canada. Managed sites are Île-du-Moine (1), Saint-Barthélemy (2), and Saint-Eugène (3); natural sites are Maskinongé Bay (A) and Yamachiche Bay (B) on the north shore and Anse-du-Fort (C) and Longue-Pointe (D) on the south shore.



plankton-type net, 0.40 m diameter, 500 µm mesh) at a velocity of 1 m·s^{-1} at depths varying between 0.5 and 2.5 m. A total of 10–54 transects of 50–600 m length were sampled in each site, depending upon the surface area and the abundance of YOY yellow perch. During the summer period in 2003, pop nets (4 m × 4 m × 1.5 m; 1 mm stretch mesh) set for 24-h periods before fishing and beach seines (12.5 m × 4 m; 3.2 mm stretch mesh) were used because larvae were larger than in 2002 and were able to escape from the push nets. After capture, larvae were immediately frozen on dry ice to prevent nucleic acid degradation and were later stored at –80 °C until laboratory analyses. Once thawed, larvae were individually identified, weighed (±0.001 g), and measured using callipers (±0.01 mm). In the case of the spring 2002 samples, owing to logistical constraints, both RNA/DNA ratios and length measurements could not be obtained on the same set of fish. Length measurements were thus obtained separately on a much larger number of fish preserved in 75% ethanol but originating from the same sampling expedition. Total length was corrected for shrinkage during preservation. For measuring the RNA/DNA ratio, whole larvae were homogenized individually in distilled water (5 *w/v*) and nucleic acids were extracted according to the method of Buckley and Bulow (1987). Nucleic acid concentrations were determined from absorbance readings at 260 nm taken on RNA and DNA fractions.

Water temperature was recorded hourly from April until August using two thermologgers (Minilog-T™, VEMCO, Shad Bay, Nova Scotia; ±0.1 °C) placed at each study site (Fig. 2). The thermologgers were protected from direct solar radiation and placed 0.2 m from the bottom of the water column.

Data analysis

Seasonal means for RNA/DNA ratio and total length were calculated for the environmental gradient represented by the three habitat types in 2002 and 2003. To determine whether the RNA/DNA ratio and total length responded similarly to the environmental gradient, Pearson's correlations between site-specific means were tested for each year, using the Bonferroni method. An analysis was also conducted to compare RNA/DNA ratios and total lengths among h.bitat types for each season (spring/summer) and year (2002/2003). Two-way analysis of variance was used followed by a Bonferroni a posteriori test. The influence of fish weight on the RNA/DNA ratio was tested by linear regression and an analysis of covariance was used to remove the effect of weight where appropriate. To test the time scale over which

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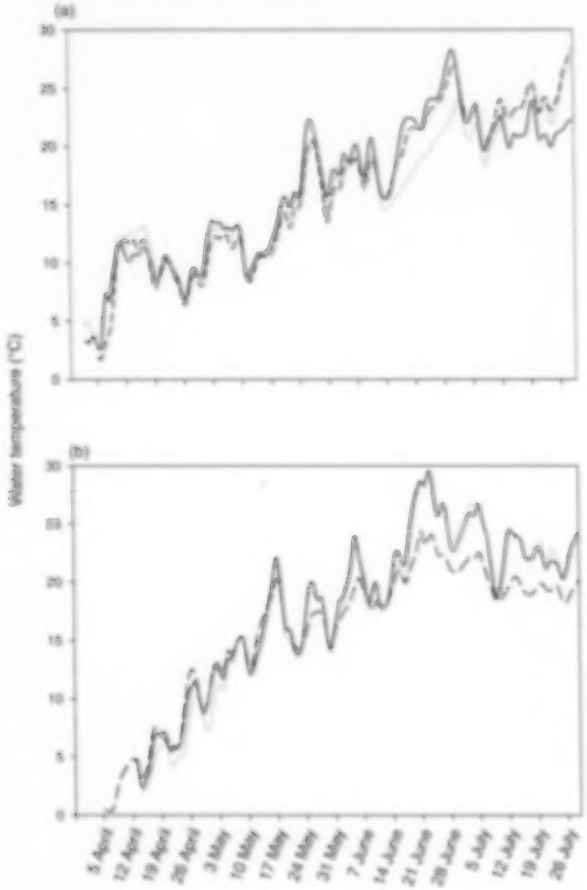
Fig. 2. Variation in water temperature from April until August at a representative managed wetland (solid line) and natural north-shore (dotted line) and south-shore (broken line) sites in Lake Saint-Pierre during 2002 (*a*) and 2003 (*b*).



each index responds to changes in temperature, linear regressions between site-specific mean RNA/DNA ratios and cumulative degree-days (water temperature >13.5 °C) were tested over short periods before sampling (1–12 days) and a long period from hatch to sampling for each year. For total length, linear regressions between site-specific means and cumulative degree-days (>13.5 °C) were tested over two time periods, from hatch to spring sampling and from hatch to summer sampling, with the 2 years combined. Cumulative degree-days above 13.5 °C were used, since 13.5 °C represents the minimal water temperature required for growth in length to occur in yellow perch (LeCren 1958; Power and Heuvel 1999). Degree-days may be more physiologically relevant to use than mean water or air temperature. The hatch date at each site was estimated from the relationship between incubation period and water temperature established by Guma'a (1978). The water temperature used was the mean of temperatures measured during the 20 days following the observed spawning date. Assumption of normality was verified and ratios were transformed when necessary using square-root and log transformations for ratios and weights, respectively. Statistical analyses were performed using SYSTAT™ version 10.0 (Systat Software Inc. 2000) and results were considered significant when $p < 0.05$.

Results and discussion

Total length versus RNA/DNA ratio

Our first aim was to compare two different types of growth indices measured in YOY yellow perch from different field habitats over seasonal and yearly temporal scales. As expected, intersite differences in length–frequency distributions, RNA/DNA ratios, and total lengths reflected the influence of the wide range of environmental conditions among the three habitat types on growth of YOY yellow perch (Figs. 3 and 4). In 2002 there was good concordance between the two indices measured over the growing season. A significant positive correlation between the RNA/DNA ratio and total length was observed in the three types of habitat ($r^2 = 0.859$, $p = 0.028$). The two indices revealed the same significant intersite differences for spring and summer, except for springtime RNA/DNA ratios between natural sites that could be explained by a small sample size from the south shore (Fig. 4). In 2003, a discrepancy between growth indices was observed for both periods studied. There was no significant correlation between indices ($r^2 = 0.530$, $p = 0.279$), and total lengths revealed the opposite trend to RNA/DNA ratios, especially for managed wetlands (Fig. 4). Previous studies have not always consistently shown a correlation between traditional and biochemical growth indices in larval and juvenile fish (Chicharo et al. 1998; Fukuda et al. 2001; Imsland et al. 2001). The discrepancy between growth in length and RNA/DNA ratio observed in 2003 could be explained by the different time scales on which these indices respond to environmental change. Rapid changes in environmental conditions should be reflected in the RNA/DNA ratio, with values increasing or decreasing over time, in contrast to total length, where size increments accumulate over time.

Temperature effects

To test the hypothesis that RNA/DNA ratios respond rapidly to changes in abiotic conditions, ratios were related to short-term (1–12 days before sampling) and long-term (from hatch to sampling) cumulative degree-days (water temperature >13.5 °C). In 2002 there was a significant positive relationship between the RNA/DNA ratio and temperature over short periods (7–8 days before sampling), while no relationship was observed over a longer period (Table 1). While temperature effects on the RNA/DNA ratio of laboratory-reared freshwater larval fish have already been examined (Malzahn et al. 2003), the importance of environmental-temperature variability during first-year growth has not. For feeding conditions, the response time of the RNA/DNA ratio is generally short and characterizes the feeding environment the larvae face within a time frame of 1 week prior to sampling (Clemmesen 1994; Bailey et al. 1995). We observed a similar response time for temperature, where cumulative degree-days measured 7–8 days prior to sampling was an important predictor of the RNA/DNA ratio. This suggest that the RNA/DNA ratio in freshwater field conditions is sensitive to

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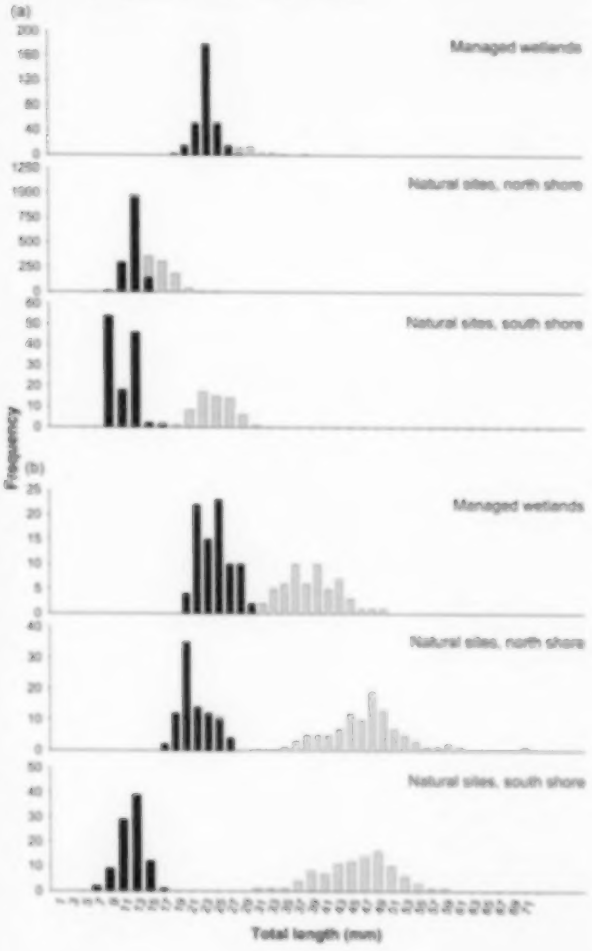
Fig. 3. Length–frequency distribution of yellow perch (*Perca flavescens*) larvae captured in managed wetlands and natural sites in Lake Saint-Pierre during the spring (solid bars) and summer (shaded bars) of 2002 (*a*) and 2003 (*b*). For 2002, $n = 313$ and $n = 33$ for managed wetlands in the spring and summer; $n = 1406$ and $n = 775$ for natural sites on the north shore in spring and summer; and $n = 121$ and $n = 63$ for natural sites on the south shore in spring and summer, respectively. For 2003, $n = 86$ and $n = 57$ for managed wetlands in the spring and summer; $n = 89$ and $n = 103$ for natural sites on the north shore in spring and summer; and $n = 92$ and $n = 97$ for natural sites on the south shore in spring and summer, respectively.



growth variations in response to short-term fluctuations of abiotic conditions. In 2002, a similarity in growth patterns between indices, coupled with a short-term temperature effect on the RNA/DNA ratio, suggest that water temperature may have been limiting to yellow perch growth during most of the early life stages. Temperature-limited growth also offers a plausible explanation of why the two growth indices responded similarly in 2002.

In 2003, no relationship between RNA/DNA ratio and cumulative degree-days were observed over either short or long periods (Table 1). In 2003, cumulative degree-days at all sites were 1.5–3.7 times higher than in 2002 (Table 2). Given that the RNA/DNA ratio responds to short-term changes in environmental conditions, the absence of a significant relationship in 2003 suggests that variables other than temperature have been limiting short-term growth. Prey availability and food quality (Post and McQueen 1994; Clapp and Dettmers 2004) and intra- and inter-cohort competition (Post et al. 1997; Romare 2000; O'Gorman and Burnett 2001) as well as interspecific competition (Persson et al. 1999; Fullerton et al. 2000) are some of the factors that limit growth of YOY yellow perch. Size-related mortality at age 0 by predation, cannibalism, or starvation can also favor larger sizes in yellow perch and may influence the size distribution at age 0 (Post and Evans 1989*b*). The discrepancy in the conclusions reached in these studies on determining factors that influence the growth of YOY yellow perch, including the present results, suggests that the limiting variables and their relative importance may shift between populations and between years.

Table 1. Linear regression of RNA/DNA ratios of yellow perch (*Perca flavescens*) on cumulative degree-days >13.5 °C water temperature for managed and natural sites in Lake Saint-Pierre.

Time period prior to sampling (days)	2002	2003
Hatch	0.500	0.235
12	0.582	0.212
11	0.568	0.227
10	0.581	0.240
9	0.613	0.230
8	0.668*	0.229
7	0.828*	0.242
6	0.648	0.243
5	0.593	0.246
4	3.103×10^{-4}	0.254
3	1.077×10^{-4}	0.261
2	0.068	0.197
1	0.010	0.257

Note: Coefficients of determination (r^2) are shown ($n = 6$); an asterisk indicates that the relationship is significant ($p < 0.05$).

For total length, a significant relationship was only found over the hatch-to-summer-sampling period with the 2 years combined ($r^2 = 0.908$, $p < 0.05$), but not over the hatch-to-spring-sampling period ($r^2 = 0.091$, $p > 0.05$) (Table 2). Temperature is known to control fish growth (Buckley 1984; Heath 1992); however, attempts to relate age-0 growth to water temperature have been more or less successful (Mooij et al. 1994; Chong 2000; O'Gorman and Burnett 2001). In 2002, even though lower temperatures were likely limiting to growth, this effect was only revealed later in the growing season. Unless enough degree-days have been accumulated, temperature may not have a significant influence on standard length. This may explain why studies do not necessarily establish a relationship between length of YOY fish and temperature when it is limiting.

Ecological implications

In this study we compare two growth indices of YOY yellow perch to better understand differences in fish productiv-

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Fig. 4. Total lengths (mean ± standard error) and RNA/DNA ratios (mean ± standard error) of yellow perch larvae captured in managed wetlands (open bars) and natural sites on the north shore (solid bars) and south shore (hatched bars) in Lake Saint-Pierre during the spring and summer of 2002 (*a*) and 2003 (*b*). For 2002, $n = 313$ for total length and $n = 85$ for RNA/DNA ratio in the spring and $n = 33$ and $n = 33$ in the summer at managed wetlands; $n = 1452$ and $n = 214$ in the spring and $n = 113$ and $n = 112$ in the summer at natural sites on the north shore; and $n = 75$ and $n = 4$ in the spring and $n = 28$ and $n = 28$ in the summer at natural sites on the south shore. For 2003, $n = 86$ and $n = 85$ in the spring and $n = 57$ and $n = 57$ in the summer at managed wetlands; $n = 89$ and $n = 88$ in the spring and $n = 103$ and $n = 103$ in the summer at natural sites on the north shore; and $n = 92$ and $n = 87$ in the spring and $n = 97$ and $n = 97$ in the summer at natural sites on the south shore. A different letter above the bar denotes a significant difference ($p < 0.05$).

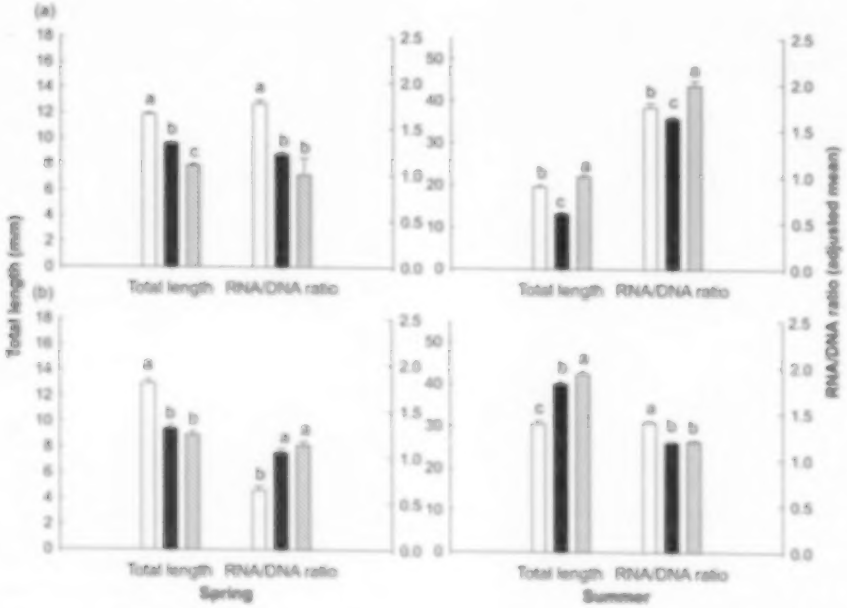

Table 2. Cumulative degree-days (>13.5 °C) and hatch dates for yellow perch in managed and natural sites in Lake Saint-Pierre in 2002 and 2003.

	Hatch to spring sampling (degree-days)[a]		Hatch to summer sampling (degree-days)[a]		Hatch date[b]	
	2002	2003	2002	2003	2002	2003
Managed sites	27	59	121	176	May 10	May 11
Natural sites						
North shore	18	59	95	354	May 8	May 13
South shore	20	54	102	326	May 12	May 15

[a]Only water temperatures > 13.5 °C were used, corresponding to those required to produce growth in length of yellow perch (Power and Heuvel 1999).
[b]Estimated from the relationship between incubation period and water temperature (Guma'a 1978).

ity processes between managed wetlands and natural habitats on a large fluvial lake with two contrasting shores. A consistent result in this study is that by springtime, larvae from managed wetlands are significantly larger and generally have a higher growth rate, as determined from total length, than those from the surrounding natural habitat (Figs. 3 and 4). This can be explained by rapid water warming associated with a stable water level and more degree-days accumulated at managed sites by springtime, particularly in cold years (i.e., 2002) (Table 2). By summertime, however, even though the temperature was higher in managed wetlands in 2002, the larvae from the natural south-shore habitats were significantly and systematically longer, reversing the pattern observed in the springtime (Figs. 3 and 4). These results suggest that variables (see the previous section) other than temperature may have influenced growth of yellow perch on the south shore. It is plausible that in managed wetlands, where inputs from surrounding rivers are minimal, decreased prey availability coupled with increasing fish densities may have become limiting to growth. This is consistent with the results of a comparison of natural-habitat and enclosure-reared YOY yellow perch, where growth variability was explained by prey availability rather than water temperature and cumulative degree-days (Post and McQueen 1994).

The implication of our results from a management point of view is that survival of larvae would likely be increased if fish produced in managed wetlands were released into natu-

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ral habitats earlier by drawdown operations: by early June, for example. Field evaluations of released hatchery-reared Japanese flounder (*Paralichthys olivaceus*) showed that juveniles with a higher RNA/DNA ratio had a higher probability of survival immediately after release in the nursery grounds (Gwak et al. 2003). Indeed, early growth can influence survival by a factor of 30 in larval fish (Rice et al. 1993), and is an important determinant of survival and recruitment for many species (Miller et al. 1988). Faster first-year growth can increase the size of age-0 yellow perch and favor over-winter survival and subsequent adult recruitment (Post and Evans 1989*a*). Higher yellow perch growth rates in spring support the contention that managed habitats have the potential to contribute to fish production in such a large fluvial lake. Still, further studies will be necessary to identify the factors limiting fish growth by summer in managed wetlands, to determine the optimal time of release into nearby habitats that most favors larval survival.

In this study we show that growth in length and the RNA/DNA ratio of YOY yellow perch on the south shore were generally higher by summer in both years (except the RNA/DNA ratio in 2003). There is good reason to believe that by summer, juveniles from Lake Saint-Pierre mostly feed on benthic organisms, since their total length corresponds to that following their migration to littoral zones (>25 mm depth) (Whiteside et al. 1985). Recent studies have established a north–south productivity gradient in Lake Saint-Pierre for organisms at low trophic levels. By summer, the growth differences found for YOY perch could be attributed to a larger quantity of benthos on the south shore of Lake Saint-Pierre, which despite being of lesser quality could support greater productivity at higher trophic levels (J.-J. Frenette, Département de chimie-biologie, Université du Québec à Trois-Rivières, C.P. 500, Trois-Rivières, QC G9A 5H7, personal communication; Huggins et al. 2004). Other studies have related significant larval growth in Japanese anchovy (*Engraulis japonicus*) (both RNA/DNA ratio and recent otolith growth) to higher primary production (Huang and Chiu 2001). The total length attained by south-shore larvae in summer supports the productivity gradient of Lake Saint-Pierre established for lower trophic levels, the south shore providing a more productive habitat than the north shore.

In conclusion, results of the present study show that total lengths and RNA/DNA ratios respond over different time scales to changes in environmental conditions. This point helped us to interpret the apparent divergence in our results. Contrasting values for the two growth indices reflected heterogeneous environmental conditions among the three study habitats and two seasons. In addition to spatial and seasonal changes, a reverse thermal pattern was observed for one of the years. Low overall temperatures recorded in 2002 likely limited growth of YOY yellow perch, while high temperatures in 2003 did not. The combination of the two indices suggests that they are complementary and that other variables such as prey availability, food quality, and competition may have an impact on growth, particularly in years with higher overall temperatures (2003). Thus, limiting variables may shift depending on environmental conditions.

The present study also supports the general contention that managed habitats are more productive than the surrounding habitat in terms of growth and represents a first step towards evaluating the importance of controlled habitats in compensating for losses due to various increasing pressures such as the sport and commercial fishery. Our results also support the existence of a spatial productivity gradient in Lake Saint-Pierre, where the south shore provides, by summertime, a more productive habitat than the north shore. Further studies should focus on gaining an understanding of temporal variation of factors other than temperature, to better understand their influence on growth at higher trophic levels.

Acknowledgements

We thank Rémi Bacon, Marie-Josée Boisvert, Denis Bourbeau, Daniel Dolan, Jean Gauthier, Kim Huggins, Bidénam Kakou, Gilles Mercier, Marie-Pascal Munger, and Yves Paradis for their conscientious work in the field and laboratory. Funding was provided through a Natural Sciences and Engineering Research Council (NSERC) research grant to H.G., a summer NSERC scholarship to D.T., and a grant from the St. Lawrence Action Plan, and support from the Ministère des Ressources naturelles et de la Faune du Québec to M.M.

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Retention of juveniles within a hybrid zone between North Sea and Baltic Sea Atlantic cod (*Gadus morhua*)

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Abstract: Elucidating the relative roles of dispersal and retention of juvenile stages is an important issue for understanding population structure and evolution in marine organisms. We investigated the genetic population structure of juvenile Atlantic cod (*Gadus morhua*) within the transition zone between the North Sea and the Baltic Sea, employing nine microsatellite loci, and compared our data with adult cod data from the same area. Small but statistically significant overall differentiation (F_{st} = 0.003) was found among juvenile samples. Samples of juveniles grouped genetically with adult samples from the same geographical regions. Individual admixture analysis of a large sample of juveniles taken within the transition zone showed that the patterns of genetic differentiation could not be explained by mixing of pure North Sea and Baltic Sea individuals. Instead, the high number of juveniles with intermediate genotypes was compatible with a scenario of exclusive local (transition zone) origin. The results support the hypothesis that population structure in marine fishes is maintained by the retention of juveniles.

Résumé : L'évaluation des rôles relatifs de la dispersion et de la rétention des stades juvéniles est une question importante pour la compréhension de la structure démographique et de l'évolution des organismes marins. Nous avons examiné la structure génétique de population de jeunes morues franches (*Gadus morhua*) dans la zone de transition entre la mer du Nord et la Baltique par l'étude de neuf locus microsatellites et nous avons comparé les résultats à ceux obtenus chez les morues adultes de la même région. Il existe une différentiation globale faible (F_{st} = 0,003), mais statistiquement significative, dans les échantillons de jeunes. Les échantillons de jeunes se regroupent génétiquement avec les échantillons d'adultes de la même région. Une analyse de mélange individuelle d'un grand échantillon de jeunes capturés dans la zone de transition montre que les patrons de différentiation génétique ne peuvent s'expliquer par un mélange d'individus purs de la mer du Nord et de la Baltique. Au contraire, le nombre élevé de jeunes à génotype intermédiaire est compatible avec un scénario d'origine exclusivement locale (zone de transition). Nos résultats appuient l'hypothèse selon laquelle la structure démographique des poissons marins est maintenue par la rétention des jeunes.

[Traduit par la Rédaction]

Introduction

Recent genetic studies of marine fishes have indicated population structure on a small geographical scale (examples in Ruzzante et al. 1998; Taylor and Hellberg 2003; Nielsen et al. 2004). The finding of marked reproductive isolation among populations of "classical" marine fish is contradictory to expectations for species that encounter few physical barriers and have wide distributions, large population sizes, high fecundity, and pelagic eggs and larvae (Gyllensten 1985; Ward et al. 1994; Waples 1998).

A number of hypotheses have been proposed to explain the occurrence of genetic differentiation among populations of marine fish, such as physical retention of eggs and larvae by oceanic currents, homing to natal spawning grounds, and selection for local adaptations associated with habitat shifts (Iles and Sinclair 1982; Palumbi 1994; Riginos and Nachman 2001). However, in most cases where microgeographical population structure has been inferred, very little is known about the relative roles of retention, homing, and selection.

A first step towards elucidating the interplay among the evolutionary forces shaping population structure in marine fishes is to investigate whether juvenile stages are retained or dispersed. Genetic methods have high potential for identification of individuals or groups of individuals originating

Received 3 December 2004. Accepted 23 June 2005. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on 16 September 2005.
J18439

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 doi: 10.1139/F05-139 © 2005 NRC Canada

Fig. 1. Map of sea areas showing sampling localities of juvenile and adult Atlantic cod (*Gadus morhua*). Samples were collected from the North Sea (1A, 1B, and 1J), Kattegat–Skagerrak (2A, 2B, and 2J), the Belt Sea (3A, 3B, and 3J), the western Baltic Sea (4A and 4J), and the Baltic proper (5A and 5B). See Table 1 for sample codes and detailed information about the samples.

from different spawning populations of fish, also in cases where individuals from different populations mix (Hansen et al. 2001; Pella and Masuda 2001). Still, discrimination among marine fish from different populations is often hampered by limited or lacking genetic differentiation (Hansen et al. 2001). Consequently, relatively little is known whether eggs, larvae, and juveniles are retained or dispersed for most species of marine fishes (but see Palumbi 1999; Palumbi and Warner 2003).

Hybrid zones are narrow regions in which genetically distinct populations meet, mate, and produce hybrids (Barton and Hewitt 1985) and have been termed windows on evolutionary process (Harrison 1990). Thus, hybrid zones provide unique opportunities to study the factors responsible for development and maintenance of population structure in marine fishes. The maintenance of a hybrid zone (i.e., a stable genetic cline within a continuous habitat) is generally best described by dispersal/selection models (Barton and Hewitt 1985), where "pure" parental individuals are dispersed into the zone and hybrid individuals selected against. Alternative dispersal-independent models such as the bounded hybrid superiority model (Moore 1977) include those where hybrids are thought to be more fit than both parental types within an ecotone. Studies of the distribution of juveniles and adults within a hybrid zone can provide evidence of the level of dispersal and in turn of relative strengths of selection and migration (Barton and Hewitt 1985; also see Gilg and Hilbish 2003 for the inference of larval dispersal investigating a *Mytilus* hybrid zone).

Previous population genetic studies of marine fishes in the transition area between the brackish Baltic Sea and the high-saline North Sea have suggested the presence of multiple hybrid zones (Nielsen et al. 2004). The area provides an ideal setting for studies of the relative distribution of juveniles and adults from different spawning populations, since interactions among populations with high levels of genetic differentiation are taking place within a narrow geographical range (Nielsen et al. 2001*a*, 2003, 2004). Also, many environmental and biological gradients are apparent in the area, and the hydrographical conditions with strong currents provide a large potential for dispersal of eggs and larvae.

In the present study, we investigated the possible origin of juvenile Atlantic cod (*Gadus morhua*) sampled in the transition zone between the North Sea and the Baltic Sea to gain further insight into the isolating mechanisms between Baltic and North Sea cod. We compared the genetic composition of juvenile cod with data on adult cod populations from the same locations (Nielsen et al. 2003). To elucidate the population of origin of individuals within samples, we used individual admixture analysis of a large sample of juveniles from the central part of the transition zone and compared this analysis with (*i*) the adult samples from the transition zone, (*ii*) simulated pure individuals from the parental populations of the North Sea and Baltic Sea, and (*iii*) simulated transition zone individuals under a random mating scenario.

Materials and methods

Sampling

A total of 187 juvenile (6–16 cm) cod were collected in 1996 from the North Sea, a transition zone area (Kattegat–Skagerrak and Belt Sea), and the Baltic Sea (see Fig. 1; Table 1). Forty individuals were from the North Sea, 47 from the Kattegat–Skagerrak, 74 from the Belt Sea, and 26 from the western Baltic Sea. Adult samples were collected in 1996 and 1997. Sample sizes varied between 40 and 88 (also see Nielsen et al. 2003 for details on adult samples). The juveniles were sampled during a standard Danish Institute for

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Table 1. Summary statistics for adult and juvenile Atlantic cod (*Gadus morhua*) samples collected showing geographic sampling area, year and month of sampling, and number of individuals collected.

Geographic locality	Sample No.	Life stage	Year	Month	No. of individuals
North Sea	1A	Adult	1996	Feb.–Mar.	82
	1B	Adult	1997	Feb.–Mar.	76
	1J	Juvenile	1996	July–Aug.	40
Kattegat–Skagerrak	2A	Adult	1996	Feb.–Mar.	50
	2B	Adult	1997	Feb.–Mar.	40
	2J	Juvenile	1996	July–Aug.	47
Belt Sea	3A	Adult	1996	Feb.–Mar.	88
	3B	Adult	1997	Feb.–Mar.	40
	3J	Juvenile	1996	July–Aug.	74
Western Baltic Sea	4A	Adult	1996	Feb.–Mar.	59
	4J	Juvenile	1996	Oct.–Nov.	26
Baltic proper	5A	Adult	1996	Apr.	74
Bornholm Basin	5B	Adult	1997	Apr.	80

Fisheries Research trawl survey covering stations from the northeastern North Sea to the western Baltic Sea. The survey stations were representative of the general bathymetry in the area, and consequently, both open sea and more shallow sheltered areas were sampled. Typically, the juveniles representing an area would be from two to four trawl hauls at different stations within that area.

Microsatellite analysis

DNA was extracted from gill tissue using a proteinase K–chelex method (Estoup et al. 1996). We employed nine highly variable microsatellite loci: *Gmo1*, *Gmo2*, and *Gmo132* (Brooker et al. 1994), *Gmo20* (Ruzzante et al. 1996*a*), *Gmo141* (Ruzzante et al. 1996*b*), and *Gmo8*, *Gmo19*, *Gmo 34*, and *Gmo37* (Miller et al. 2000). Standard polymerase chain reaction reagents were used and the microsatellites were subsequently analysed on a Pharmacia ALFexpress® automated sequencer using the procedures recommended by the manufacturer.

Statistical analysis

The program Fstat (Goudet 1995) was used to test for deviations from Hardy–Weinberg and gametic phase equilibrium and to estimate pairwise F_{st} values between all samples using Weir and Cockerham's (1984) statistics and for testing their significance. Multidimensional scaling analysis of D_A distances of Nei et al. (1983) was conducted using ViSta 5.6.3 (Young 1996) to visualize genetic relationships among samples. The program STRUCTURE (Pritchard et al. 2000) was used to estimate individual admixture proportions (i.e., the estimated proportion of an individual's genotype originating from one or the other of the parental populations). STRUCTURE is a model-based Bayesian, Markov chain Monte Carlo approach that clusters individuals to minimize Hardy–Weinberg disequilibrium and gametic phase disequilibrium between loci within groups. Individual admixture proportions (q) were calculated by assuming an admixture model (i.e., allowing the genetic composition of individuals to be a mixture from different populations). The model was forced to consider the individuals from two baseline samples, North Sea (pooled North Sea 1996 and 1997 adults) and Baltic proper (pooled 1996 and 1997 Bornholm Basin adults), as pure (i.e., nonadmixed individuals). Allele frequencies in populations were assumed to be correlated (see Nielsen et al. 2003 for more details on the analysis). Simulated individuals based on allele frequencies of the adult samples from the North Sea, Baltic Sea, and Belt Sea were used to generate 90% confidence intervals of individual admixture proportions (1000 from each sea area). The simulated individuals were created by the program HYBRIDLAB (Nielsen et al. 2001*b*) (the program is available upon request from the corresponding author). The program generates simulated multilocus genotypes (hybrids and parentals) by drawing alleles with a probability equal to their estimated frequency from population samples. Hybrids are generated by drawing one allele at each locus from both parental populations and parentals are generated by drawing two alleles at each locus from the same population. We used a Kolmogorov–Smirnov two-sample test to evaluate differences between distributions of individual admixture proportions in observed and simulated samples.

Results

One significant deviation (after Bonferroni correction) from Hardy–Weinberg expectations was found for locus *Gmo 2* in the sample from the Belt Sea. No significant genotypic disequilibrium was found for any pair of loci in any of the samples of juveniles. (For results from adult samples see, appendix 1 and table 4 in Nielsen et al. 2003). Small (F_{st} = 0.003) but significant (p = 0.028) overall differentiation was found among juvenile samples. Estimates of pairwise genetic differentiation among juvenile samples were small and nonsignificant, ranging from 0.001 between the North Sea and Skagerrak–Kattegat to 0.009 between the North Sea and western Baltic Sea (see Appendix A). All juvenile samples were significantly different from adult Baltic Sea (western Baltic and Baltic proper) samples. Juveniles from the Belt Sea and western Baltic Sea were significantly different from adult North Sea cod. Samples of juveniles grouped genetically with adult samples from the same geographical region in the multidimensional scaling plot (Fig. 2) of dimensions 1 and 2 (explaining 68% and 20% of the variance, respectively). An exception was the sample of juveniles from the western Baltic Sea (4J), which grouped closer to (and was not significantly differentiated from) Belt Sea

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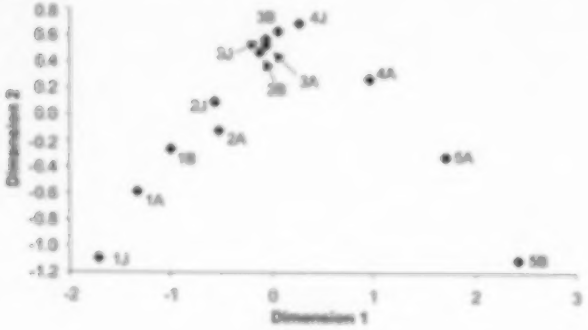
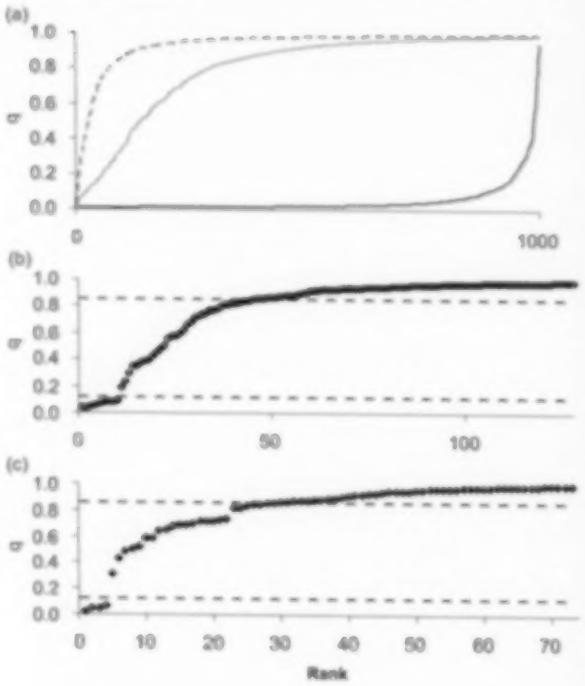
Fig. 2. Multidimensional scaling plot of D_A distances of Nei et al. (1983) among samples of juvenile and adult Atlantic cod (*Gadus morhua*). Numbers refer to the sample codes listed in Table 1.



adult samples (3A + 3B) than to the adult sample from the western Baltic Sea (4A), from which they were also significantly differentiated. The juveniles from the North Sea (1J) appeared not to group tightly with the adult North Sea samples (1A and 1B); however, in this case, there was no significant pairwise differentiation. The distribution of individual admixture proportions of Belt Sea juveniles (Fig. 3*c*) was very similar to, and not significantly different from, the distribution among adult (Fig. 3*b*) and simulated Belt Sea cod (Fig. 3*a*) ($p = 0.577$ and 0.411, respectively) but different from both simulated North Sea ($p < 0.001$) and simulated Baltic Sea cod ($p < 0.001$) (Fig. 3*a*). A relatively high number of juvenile and adult Belt Sea cod showed intermediate genotypes outside the 90% confidence interval of pure North Sea and pure Baltic proper cod. This proportion (23/74) was significantly (χ^2, $p < 0.005$) higher than the 10% (8/74) expected in a scenario of mechanical mixture of pure individuals from the parental populations alone.

Fig. 3. Distribution of q (admixture proportion) values. The values have been ranked starting from pure Baltic Sea Atlantic cod (*Gadus morhua*) (a value of 0) to pure North Sea cod (a value of 1): (*a*) q values of simulated individuals created from the allele frequency distributions of North Sea (broken line), Belt Sea (thinner solid line), and Baltic proper (thicker solid line) adult samples (1000 for each); (*b*) true samples of adults from the Belt Sea; (*c*) pooled juvenile samples (3A and 3B) from the Belt Sea. For Figs. 3*b* and 3*c*, 90% confidence intervals of admixture proportions for simulated pure North Sea and pure Baltic Sea individuals are indicated.



Discussion

Origin of juveniles

The data show a clear association between juveniles and adult samples collected at the same location in the transition area between the North Sea and the Baltic Sea. Similarly, the admixture analyses show that there are a number of individuals in the Belt Sea sample with genotypes unlikely to be of pure Baltic Sea or North Sea origin, whereas their genotypes are congruent with recruitment from local adult spawners. Accordingly, the most parsimonious explanation of the data is predominantly local origin of juveniles. On the other hand, we cannot rule out the possibility of occurrence of some pure North Sea or Baltic Sea individuals for any of our samples of juveniles owing to lack of statistical power for unambiguous individual assignment. In particular for the samples collected adjacent to the parental North Sea and Baltic proper populations (i.e., western Baltic Sea and Skagerrak–Kattegat), the only possible evidence is the genetic relationship among juvenile and adult samples from the same area calculated on the basis of the total sample. Likewise, individuals of western Baltic Sea and Skagerrak–Kattegat origin cannot be identified in the Belt Sea sample.

The only juvenile sample that did not group tightly with the associated adult sample was the sample from the western Baltic Sea. This could be caused by dispersal from the Belt Sea into the western Baltic Sea population. However, as mentioned previously, pairwise differentiation among juvenile samples was not significant, so this could in part be caused by the small population sample size and generally low levels of differentiation. Further, there is only one adult sample from the western Baltic Sea, so we have no indications of the temporal variability of the genetic composition in that area. Still, the lack of clear association with the adult western Baltic Sea sample could be caused by dispersal of juveniles from the Belt Sea into the western Baltic Sea population. Larval drift from the Belt Sea to the western Baltic Sea is supported by analysis of otolith microstructure (Oeberst 2001). He suggested that juveniles spawned in the Belt Sea drift into the western Baltic Sea and can occur even among eastern Baltic Sea cod (i.e., Bornholm Basin), thus illustrating spatial and temporal variability in the patterns of dispersal. Along the same lines, Knutsen et al. (2004) re-

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ported that dispersal of juvenile cod of North Sea origin into coastal Skagerrak populations varied between years.

Juvenile retention and oceanography

The reasons for limited juvenile dispersal are at this moment not well known. Naturally, limited dispersal can have purely oceanographic origin, but several authors have indicated that juvenile behavior can be important to avoid dispersal (see Riginos and Victor 2001; Palumbi and Warner 2003). Also, Taylor and Hellberg (2003) found that the pattern of differentiation among Caribbean reef fish was not well predicted by pure oceanography and duration of larval phase. In the case of dispersal of cod eggs and larvae between the North Sea and the Baltic Sea, there should be ample opportunity for dispersal during the egg and larval stage owing to the strong currents found in the area. However, the net effect of these currents with regard to dispersal of cod eggs, larvae, and pelagic juveniles between the pure Baltic Sea and North Sea populations could be limited owing to the vertical distribution of the early life stages of cod in relation to the salinity profile of the flowing water. In the northern Belt Sea, there is a sharp cline (pycnocline) between highly saline North Sea water close to the bottom and low-saline Baltic Sea water at the surface. The neutral buoyancy of North Sea and Skagerrak–Kattegat cod eggs, which is above 20 practical salinity units (psu) (Nissling and Westin 1997), means that cod larvae are distributed below the pycnocline in the northern Belt Sea (K. Sünksen, K.K. Nielsen, and P. Grønkjær, unpublished data). Accordingly, North Sea cod are likely to be transported into the southern Belt Sea and Baltic Sea only during very strong inflows of high-saline North Sea water. Similarly, the eggs of the eastern Baltic Sea cod are confined to deeper water masses in the Baltic basins with a relatively high salinity and therefore not advected with the very low saline surface waters out into the Skagerrak–Kattegat (Bagge et al. 1994). In contrast, it is more likely that eggs and larvae will be exchanged between the areas within the transition zone (i.e., the Belt Sea and western Baltic Sea), because this area constitutes a more continuous spawning area with intermediate salinity and egg buoyancy (Nissling and Westin 1997). In line with this, a modelling exercise by Hinrichsen et al. (2001) showed substantial drift of eggs and larvae from the southern Belt Sea into the Arkona Basin in the western Baltic Sea. This drift occurred during both years examined (1993 and 1998) despite the fact 1998 was characterized by very low and variable wind conditions and consequently low movement of water masses relative to more normal years. In short, the hydrographical conditions and the biological characteristics of the eggs and larvae suggest that mechanical mixing of early life stages between Skagerrak–Kattegat–North Sea and the Baltic Sea could be a rare event, whereas mixing of Belt Sea and western Baltic Sea may occur even during meteorological conditions that are likely to facilitate retention.

Juvenile retention and hybrid zone maintenance

It is apparent that the dispersal of juveniles from the parental population into the center of the hybrid zone is low, or alternatively, very strong selection takes place against parental (North Sea and Baltic proper) genotypes at early juvenile stages (egg and larvae) within the hybrid zone. In the latter case, the pattern conforms to a dispersal-independent hybrid zone like the bounded hybrid superiority model (Moore 1977) (i.e., hybrid cod found within the ecotone between the North Sea and Baltic Sea (Skagerrak–Kattegat, Belt Sea, and western Baltic Sea)) should be more fit than parental genotypes and less fit outside the ecotone (North Sea and Baltic proper). In relation to a dispersal–selection model, selection against hybrids should apparently be low, since the majority of offspring are produced by local hybrid adults, and hence, dispersal of parental (North Sea and Baltic proper) juveniles into the zone is low. Generally, the level of linkage disequilibrium can be used to shed light on the forces responsible for maintenance of a hybrid zone. That is, strong correlations of gene identities across loci suggest a high rate of dispersal of parental genotypes into the center of the zone associated with strong selection against hybrid genotypes (i.e., a dispersal–selection model). However, since selection is apparently low no matter whether the zone is maintained by hybrid superiority or inferiority, analysis of linkage disequilibrium does not provide additional insight (also see Nielsen et al. 2003).

Interactions among populations

Ecological data on adaptive differences of Belt Sea and Baltic Sea cod could provide clues as to how and where interactions between North Sea and Baltic Sea cod take place. In a "reciprocal transplant" experiment, Nissling and Westin (1997) found that sperm mobility and egg buoyancy were inherited traits, with trait values differing between individuals sampled in the Belt Sea and Baltic proper. Activation of the spermatozoa occurred at and above 11–12 psu for Baltic Sea cod and at 15–16 psu for Belt Sea cod. Neutral egg buoyancy was obtained at 14.5 ± 1.2 and 20–22 psu, respectively. This means that North Sea and Belt Sea male cod will not be able to reproduce in the Baltic Sea, and the eggs of females will sink. In the North Sea and Belt Sea, Baltic Sea male cod can reproduce, but the eggs of females will float. The authors speculated that the intermediate conditions for reproduction in the western Baltic Sea would constitute an area of potential interaction. Still further "common garden" experiments of North Sea, Baltic Sea, and their hybrids in different environments are necessary to understand this system in detail. Many other confounding selective agents might still add to the complexity of the interaction.

Potential role of homing

Homing to natal spawning grounds is not necessary to explain the maintenance of population structure in the transition area but could act in parallel to increase isolation. Homing of juveniles to natal areas could of course be an alternative but not very likely explanation. However, the relative role of retention and dispersal with subsequent homing may vary with respect to a population. In a recent review, Robichaud and Rose (2004) suggested four different types of migratory behavior of adult Atlantic cod, sedentary, accurate homers, inaccurate homers, and dispersers, based on a century of physical tagging data. This is of course not necessarily representative for juvenile dispersal but merely demonstrates the potential for ecological diversity among populations.

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Juvenile retention and fisheries management

The finding of local origin of juveniles does not only have implications for our understanding of the evolution of marine fish populations but can also provide recommendations for the sustainable management of local fish stocks. In this particular case, it is apparent that the local recruitment to the fishery and spawning population can be extremely important and accordingly should be reflected in the management of cod within the transition zone between the North Sea and the Baltic Sea.

Acknowledgements

We thank Finn Sivebæk for help with the illustrations, Anders Koed for assistance with the statistical analysis, Dorte Bekkevold and Michael M. Hansen for inspiring comments, and the Danish Ministry of Food, Agriculture, and Fisheries for financial support.

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Appendix A

Table A1. Estimates of pairwise genetic differentiation (F_{st}) among Atlantic cod (*Gadus morhua*) samples, 95% confidence intervals (below the diagonal), and *p* values (above diagonal).

Sample	1A	1B	1J	2A	2B	2J	3A	3B	3J	4A	4J	5A	5B
1A	—	0.1092	0.3388	0.0084	0.0957	0.3091	0.0001	0.0019	0.0007	<0.0000	0.0210	<0.0000	<0.0000
1B	0.004	—	0.0959	0.0011	0.4030	0.4898	0.0159	<0.0000	0.0064	<0.0000	0.0091	<0.0000	<0.0000
1J	0.002	0.001	—	0.0433	0.5228	0.1517	0.0004	0.0064	0.0808	<0.0000	0.3181	<0.0000	<0.0000
2A	0.004	0.002	0.003	—	0.8001	0.3661	0.2809	0.0110	0.0557	<0.0000	0.1158	<0.0000	<0.0000
2B	0.008	0.000	0.002	0.000	—	0.9468	0.9503	0.7132	0.8308	0.0001	0.6193	<0.0000	<0.0000
2J	0.005	0.000	0.001	0.002	0.000	—	0.1447	0.0180	0.5734	<0.0000	0.0865	<0.0000	<0.0000
3A	0.006	0.003	0.006	0.000	0.000	0.002	—	0.7763	0.8164	<0.0000	0.3147	<0.0000	<0.0000
3B	0.005	0.005	0.007	0.001	0.000	0.004	0.000	—	0.2604	0.0059	0.5692	<0.0000	<0.0000
3J	0.006	0.003	0.004	0.001	0.000	0.000	0.000	0.000	—	<0.0000	0.3579	<0.0000	<0.0000
4A	0.026	0.021	0.025	0.016	0.010	0.017	0.010	0.008	0.010	—	0.0006	0.0082	0.0030
4J	0.008	0.011	0.009	0.005	0.004	0.006	0.003	0.002	0.001	0.008	—	<0.0000	<0.0000
5A	0.053	0.049	0.060	0.043	0.035	0.044	0.031	0.029	0.031	0.007	0.027	—	0.2248
5B	0.043	0.040	0.047	0.036	0.027	0.036	0.026	0.021	0.024	0.003	0.021	0.000	—

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Environmental predictors of benthic consumers and autotrophic communities along a recovery gradient

Kristy L. Hogsden and Rolf D. Vinebrooke

Abstract: Ecological theory predicts that biological factors replace abiotic regulation of community structure during recovery from ecosystem stress. We examined relationships between benthic autotroph (epilithic periphyton) and consumer communities, and environmental variables, along a gradient of six recovering acidified lakes to identify the best explanatory variables of community structure. Dissolved organic carbon, pH, and total dissolved phosphorus were important predictors of autotrophic biomass, while total dissolved phosphorus was the only significant factor explaining variation in consumer biomass. Abiotic factors (e.g., dissolved organic carbon, pH) were also significant predictors of autotrophic and consumer community composition. Autotrophic biomass was significantly greater in recovering lakes owing to an increased abundance of attached filamentous green algae. However, consumer biomass did not differ significantly between severely stressed and recovering lakes because of a compensatory shift from numerous small tolerant omnivores to fewer large-bodied sensitive grazers. Lack of a significant relationship between autotrophic and consumer biomass along with stable isotopic evidence of few primary consumers suggested that grazing pressure was weak, especially in the stressed lakes. The persistent importance of abiotic factors to autotrophic and consumer communities suggested that ecosystem recovery remained incomplete in these lakes.

Résumé : La théorie écologique prédit que les facteurs biologiques remplacent les facteurs abiotiques dans le contrôle de la structure de la communauté lorsque l'écosystème récupère d'un stress. Nous avons examiné les relations entre les communautés benthiques d'autotrophes (périphyton épilithique) et de consommateurs et les variables du milieu sur un gradient formé de six lacs acidifiés en récupération pour déceler les meilleures variables explicatives de la structure de communauté. Le carbone organique dissous, le pH et le phosphore dissous total sont des variables prédictives importantes de la biomasse des autotrophes, alors que seul le phosphore dissous total est un facteur explicatif significatif de la variation de la biomasse des consommateurs. Les facteurs abiotiques, tels que le carbone organique dissous et le pH, sont aussi des variables prédictives significatives de la composition des communautés d'autotrophes et de consommateurs. La biomasse des autotrophes est significativement plus importante dans les lacs en récupération à cause de l'abondance accrue des algues vertes filamenteuses attachées. Cependant, la biomasse des consommateurs ne diffère pas significativement entre les lacs fortement stressés et les lacs en récupération à cause d'un remplacement compensatoire des nombreux petits omnivores tolérants par un nombre moindre de brouteurs sensibles de grande taille. L'absence de relation significative entre la biomasse des autotrophes et celle des consommateurs, de même que le petit nombre de consommateurs primaires (confirmé par l'analyse des isotopes stables), laissent croire que la pression de broutage est faible, particulièrement dans les lacs stressés. L'importance persistante des facteurs abiotiques pour les communautés d'autotrophes et de consommateurs semble indiquer que la récupération est encore incomplète dans ces lacs.

[Traduit par la Rédaction]

Introduction

Abiotic and biotic factors affect ecosystem recovery from chemical stress (Yan et al. 2003). In particular, biotic factors expectedly supersede abiotic regulation of communities as species and trophic interactions become reestablished during ecosystem recovery (Menge and Sutherland 1987). For example, grazing is expected to increasingly regulate benthic autotrophs (e.g., epilithic periphyton) as chemical conditions ameliorate during abiotic recovery from anthropogenic acidification (Vinebrooke 1996; Vinebrooke et al. 2003*a*).

Autotrophic microbes are very responsive to environmental changes in lakes owing to their wide dispersal potential, fast growth rates, high fecundity, and emergence from sedimentary resting structures (Hansson 1996; Finlay 2002). For example, significant associations between benthic autotrophs and changes in acidity and dissolved organic carbon (DOC) occur during the early stages (pH < 6) of recovery from

Received 12 November 2004. Accepted 11 April 2005. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on 16 September 2005.
J18409

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 doi: 10.1139/F05-138 © 2005 NRC Canada

acidification (Vinebrooke et al. 2003*a*). However, autotrophic responses to abiotic improvements become variable within and between lakes at pH > 6 (Vinebrooke et al. 2003*a*). A potential explanation for such discrepancies is that these study lakes had different ecological histories (Fischer et al. 2001). Specifically, differences in recolonization by grazers could explain variation in autotrophic communities between lakes.

Many benthic consumers are capable of active aerial dispersal (e.g., aquatic insects), enabling them to track environmental changes in lakes (Jackson and Harvey 1993; Carbone et al. 1998; Bohonak and Jenkins 2003). In contrast, amphibians, fish, and invertebrates lacking aerial adult stages (e.g., amphipods, snails) are slow to respond to chemical recovery, particularly in lakes that lack migratory routes connecting them to remnant populations in neighboring lakes (Jackson and Harvey 1993; Mills et al. 2000). However, recolonization by aquatic insects can also lag behind chemical recovery owing to dispersal limitation or predation (Bendell and McNicol 1995; Carbone et al. 1998; Snucins 2003), thereby weakening relationships between benthic consumers, autotrophs, and the abiotic environment.

Ecosystem stress expectedly causes size shifts in biological communities because smaller organisms are generally more stress tolerant (Odum 1985; Cattaneo et al. 1998). In stressed lakes, size shifts are associated with changes in taxonomic and functional composition. Size reductions have been observed at the species level (Cattaneo et al. 1998) and community level (Bendell and McNicol 1995). Conversely, a size shift towards larger sensitive species should occur during ecosystem recovery, but supporting evidence from recovering stressed aquatic ecosystems is limited (but see Rask et al. 2001; Cattaneo et al. 2004).

The purpose of this study was to quantify relationships between environmental factors and the abundance and taxonomic composition of autotrophs and benthic consumers in six recovering acidified boreal lakes. Our main objective was to test the hypothesis that biotic relationships between autotrophic communities and benthic consumers had become stronger than previously reported (Vinebrooke 1996; Vinebrooke and Graham 1997) as a result of an additional 10-year period of ecosystem recovery in the study lakes. Also, we expected that benthic consumer communities would show a size shift towards larger taxa along the recovery gradient.

Methods

Study area

The survey lakes were located in Killarney Provincial Park (46°3′N, 81°21′W), a 48 000-ha wilderness area southwest of Sudbury, Ontario, Canada. Killarney Park was one of the first areas in Canada where the damaging effects of acidification were identified. As a result of spatial proximity to sulfide ore metal smelters in Sudbury and being located in an area of high deposition of long-range pollutants, the history of acidification in the region is well documented (e.g., Beamish and Harvey 1972; Dixit et al. 1992). Reduced industrial emissions of sulfur have lead to chemical and limited biological recovery in many lakes in the region (Keller et al. 1992; Snucins et al. 2001).

Survey lakes were selected nonrandomly based on pH, a key determinant of recovery from acidification (Keller et al. 2003). These lakes were chosen from past limnological surveys (Vinebrooke 1996; Vinebrooke and Graham 1997; Snucins et al. 2001) of the area to be representative of the gradient of chemical recovery occurring within the park. We restricted our survey to intensive monthly sampling of three severely acidified lakes and three circumneutral lakes because the degree of confidence in biological measurements along an environmental gradient is lowest at both ends of the grade (Vinebrooke and Graham 1997). Therefore, the main focus of our survey was to identify and contrast major differences between biological parameters in severely acidified and recovering circumneutral lakes rather than detect patterns along the entire gradient of recovering lakes in Killarney Park.

The two groups of surveyed lakes differ substantially in their catchment characteristics. The three acidified lakes chosen were Acid, Ruth Roy, and Norway. Their high-elevation catchments consist mainly of exposed orthoquartzite bedrock and northern mixed forest, which is typical of other severely acidified lakes in the park (Gunn et al. 2001). In contrast, the three recovering lakes (A.Y. Jackson, Carlyle, and Little Sheguiandah) are located in lowland areas with limestone, diabase, or sandstone bedrock with a higher buffering capacity (Snucins et al. 2001). These catchments are dominated by northern mixed forest, exposed bedrock, and some small wetlands, which characterize many of the other relatively well-buffered lakes in the area. As a result, the two lake groups differed in terms of morphology, current water chemistry, and acidification history (Table 1) and functioned as independent samples because they were not physically connected to each other. Two of the highly acidified lakes (Acid and Ruth Roy) were fishless, while the other (Norway) contained sparse populations of central mudminnows (*Umbra limi*) and yellow perch (*Perca flavescens*) (E. Snucins and J. Gunn, Cooperative Freshwater Ecology Unit, Department of Biology, Laurentian University, Sudbury, ON P3E 2C6, Canada, unpublished data). The three recovering circumneutral lakes contained yellow perch and smallmouth bass (*Micropterus dolomieu*) along with brown bullhead (*Ameiurus nebulosus*) and pumpkinseed (*Lepomis gibbosus*) in Carlyle and Little Sheguiandah.

Lake water chemistry

Water samples were collected once a month (May–August 2003) from the littoral area within each lake, concurrent with autotroph and consumer sampling, and were analyzed for pH, conductivity, total nitrogen (TN), total dissolved phosphorus (TDP), DOC, and dissolved inorganic carbon (DIC) analysis. Lake pH was measured using an Accumet AP61 portable pH meter (Fischer Scientific) on the collection date. Duplicate monthly samples from each lake were analyzed at the Limnology Laboratory at the University of Alberta (Edmonton, Alberta, Canada). In the laboratory, pH was measured on a Fisher Accumet model 925 meter and was compared with values recorded during summer sampling. Conductivity was measured using a Radiometer/ Copenhagen model CDM83 meter. TN samples were predigested with alkaline potassium persulfate according to Ameel et al. (1993) and determined by flow injection analysis on a Technicon™ Autoanalyzer™.

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Table 1. Water chemistry, morphometry, and acidification histories for six Killarney Park lakes.

Lake	Date of survey	pH	Conductivity (μm·s^{-1})	TN (μg·L^{-1})	TDP (μg·L^{-1})	DOC (mg·L^{-1})	DIC (mg·L^{-1})	Lake surface area (ha)	Mean depth (m)	Maximum depth (m)	Acidification history (pH) 1971–1973	1992	2003
Stressed													
Acid	0	5.11	20	273.51	1.8	1.7	0.69	19.6	10.9	29	—	5.1	5.1
	30	5.36	19.8	209.97	3.5	2.17	0.77						
	60	5.04	19.2	198.59	0.6	2.06	0.43						
	90	5.04	19.7	244.76	1.9	2.32	0.73						
Norway	0	5.2	23.3	250.73	2.3	2.16	0.45	63.3	15.1	33.6	4.7	4.5	5.4
	30	5.41	28.6	296.8	—	2.43	0.65						
	60	5.46	22	206.67	0.7	1.86	0.48						
	90	5.55	26.4	239.08	—	1.71	0.54						
Ruth Roy	0	6.89	49.7	273.95	4.2	3.22	3.69	54.5	4.3	18	4.5	4.2	5.8
	30	6.37	30.2	304.81	1.8	1.9	1.41						
	60	4.85	22.3	264.22	0.9	1.55	0.35						
	90	4.91	21.1	217.91	1.6	1.17	0.37						
Mean		5.43	25.19	248.42	1.93	2.02	0.88	45.8	10.1	26.9	4.6	4.6	5.4
SE		0.18	2.46	10.23	0.35	0.15	0.27	13.4	3.1	4.6	0.1	0.3	0.2
Recovering													
AY Jackson	0	5.65	15.3	232.52	1.2	1.98	0.57	6.5	7.0	9.8	5.0	5.6	6.2
	30	6.8	23.8	304.82	—	3.16	0.91						
	60	6.12	23.1	316.87	1.8	3.26	0.75						
	90	6.01	23	273.51	1.4	3.5	0.67						
Carlyle	0	7.14	38.9	379.24	—	3.74	2.23	156.7	5.7	14.6	5.0	5.1	6.3
	30	5.96	24.9	329.69	—	4.31	0.59						
	60	6.19	27.1	278.56	2.7	4.18	0.61						
	90	5.75	19	250.13	—	4.19	0.58						
Little Sheguiandah	0	6.65	34.9	351.48	5.5	4.14	1.7	4.5	1.7	2.7	5.0	6.3	6.9
	30	7.35	37.2	323.08	6.5	5.16	2.64						
	60	6.48	26.8	331.92	2	4.82	1.33						
	90	6.91	—	314.1	2	4.65	1.83						
Mean		6.42	26.73	307.16	2.89	3.92	1.2	55.9	4.8	9.0	5.0	5.7	6.5
SE		0.16	2.24	12.15	0.66	0.25	0.21	50.5	1.6	3.5	0.0	0.3	0.2

Note: Water chemistry is based on samples taken from the littoral area over a 90-day survey period. Lakes are arranged in order of decreasing acidity. Acidification history data (1971–1973 and 1992) were taken from Vinebrooke and Graham (1997) and 2003 data represent mean values from the survey period. —, data not available.

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TDP was measured using standard colorimetric procedures following digestion with potassium persulfate (Menzel and Corwin 1965). DOC and DIC were both analyzed using a Shimadzu TOC-5000A analyzer with an ASI 5000A autosampler (Shimadzu Corporation, Kyoto, Japan).

Survey of benthic autotrophs

The survey was conducted monthly, on dates corresponding to zoobenthos collections, to characterize the autotrophic community on rocks (epilithon) in all six lakes. Five rocks were collected from the littoral area of each lake and placed in a white porcelain tray. Periphyton was brushed from the upper surfaces of the rocks using a hard-bristled toothbrush, rinsed with filtered lake water, and pooled. The sampled surface area of the rocks was measured using foil wrap and recorded (Aloi 1990). Samples were visually inspected to remove large particulate contaminants or invertebrates. The harvested material was transferred from the tray to a 500-mL graduated cylinder and was filled to 200 mL with filtered lake water. A 50-mL subsample was filtered and concentrated on Whatman GF/C glass microfiber filter papers and frozen for stable isotope analysis. A 100-mL subsample was preserved with Lugol's solution for taxonomic analysis. Samples were well mixed and large filaments were broken apart using scissors and diluted depending on the density of autotrophic and detrital material before settling for 1 h using the Utermöhl method (Wetzel and Likens 2000). Species lists were constructed for each sample by scanning the chamber, starting at a low magnification (200×) for large colonies and filaments and then up to 400× and 1000× for all other taxa on a Leica DM-IRB inverted microscope. A minimum of 300 viable cells were identified and counted per sample, with cell viability based on chloroplast integrity for eukaryotic cells and pigmentation for cyanobacteria. Taxonomic identifications were made to genus level according to Prescott (1982) and Wehr and Sheath (2003). Duplicate counts of each sample were done, and if species richness (i.e., number of species) varied by more than 20%, a triplicate sample was counted.

Cell counts were converted to areal biovolumes using standard geometric formulae for shapes that approximate algal shapes (Hillebrand et al. 1999; Sun and Liu 2003). Biovolume is considered the most precise measure of algal abundance (Morin and Cattaneo 1992) and was estimated on a per-cell basis whether the cells were solitary or filamentous. A minimum of 20 cells per taxon was used to determine its mean dimensions when sufficient cell numbers were available. Calculations corrected for differences in sample surface area, enumeration area, magnification, cell size, density, and dilutions:

$$(1)\quad \text{Taxon areal abundance } (\text{mm}^3/\text{m}^2) = \frac{\text{no. of algal units counted}}{\text{mL of sample surveyed}} \times \text{dil } 1 \times \frac{\text{mL of total sample}}{\text{m}^2 \text{ of tile scraped}} \times \text{dil } 2 \times \frac{\text{mm}^3}{\text{algal unit}}$$

where mL of sample surveyed = surface area of counting cell × mL of sample settled, dil 1 = sample dilution factor prior to counting (10 or 40 times), and dil 2 = dilution factor for mL of total sample taken (200 mL taken in field, 100 mL for taxonomic analyses). Algal biovolumes were converted to biomass assuming a specific gravity of 1.

Collection of detritus

Detrital material collected from D-net sweeps was used to characterize the nearshore particulate organic material (POM) in each lake. POM was filtered through a 150-µm sieve to separate coarse particulate organic material (CPOM) and fine particulate organic material (FPOM). FPOM was settled and filtered on to a Whatman GF/F glass microfiber filter (0.7-µm mesh size). All POM samples were visually inspected to remove large particulate contaminants or invertebrates. Duplicate samples were collected from each lake and frozen until stable isotope analysis.

Survey of benthic consumers

Zoobenthos in each lake were sampled monthly from June to August 2003. Samples from the shallow littoral (depth <1 m) were collected using a D-frame kicksweep net (500-µm mesh size). To account for habitat heterogeneity, three time-standardized D-net sweeps were done in three different habitat areas for 3 min. Samples were pooled, stored in coolers, and sorted within 6 h. Large predators were separated (i.e., stored in different container) from the samples until sorting to avoid consumption of prey.

All consumers were sorted taxonomically to family and then frozen until stable isotopic analyses could be performed. Invertebrates were examined under a dissecting microscope at 40× and 100× magnifications. Many invertebrates were collected as early instars and were difficult to identify to genus or species; therefore, family level was used for all statistical analyses. All consumers were also measured for size at the widest part of the body (usually head or abdomen) and classified according to published dietary descriptions and functional-feeding groups (Merritt and Cummings 1984; Thorp and Covich 2001). Of the 23 consumer families collected, nine were classified as grazers that feed primarily on algal, plant, or detrital material, nine were classified as omnivores, and five other families were classified as predators (Table 2). After drying at 60 °C for 96 h, samples were weighed. Dry weight for snails and crayfish was shell free. Samples were then prepared for stable isotope analyses.

Stable isotope analyses

Autotroph, POM, and benthic consumer samples (i.e., whole invertebrates and amphibian and fish tissue) were frozen, dried at 60 <C for 48 h (autotroph, POM) and 72 h (consumers), and ground into a fine powder using a mortar and pestle. Dry samples were packed into 8 mm × 5 mm tin capsules for isotopic analyses. Autotroph and POM samples were peeled from filter papers prior to packing. Isotope ratios are expressed in delta (δ) notation defined as parts per thousand (‰) deviation from a standard reference material:

$$(2)\quad \delta X = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$$

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Table 2. Taxonomic composition of surveyed consumers present (+) in stressed and recovering lakes.

No.	Order	Family	Functional group	Stressed	Recovering
1	Amphipoda	Hyallidae	G	+	+
2		Gammaridae	G		+
3	Anura	Ranidae	G		+
4	Coleoptera	Dytiscidae, adult	O	+	+
5		Dytiscidae	O	+	
6		Gyrinidae	O	+	
7	Cypriniformes	Cyprinidae	O		+
8	Decapoda	Cambaridae	O	+	+
9	Diptera	Chironomidae	O	+	+
10	Ephemeroptera	Ephemerellidae	G		+
—[†]		Heptageniidae	O	+	+
—[†]		Leptophlebiidae	G		+
11	Gastropoda	Viviparidae	G		+
12	Hemiptera	Corixidae	O	+	
13		Notonectidae	O	+	+
—[†]	Hydrachnida	Unknown	P	+	+
14	Isopoda	Asellidae	G[*]	+	+
—[†]	Megaloptera	Corydalidae	P	+	+
—[†]		Sialidae	P		+
15	Odonata (Anisoptera)	Libellulidae	P	+	+
16		Gomphidae	P	+	+
17	Odonata (Zygoptera)	Coenagriondae	P	+	+
18	Trichoptera	Limnephilidae	G	+	+
19		Unknown	G	+	+

Note: Functional group affiliation for each family is listed (G, grazer; O, omnivore; P, predator). Taxon identification numbers correspond to those used in the CCA plots.

[*]Primarily feeds on detritus.

[†]Excluded from CCA.

where X represents $\delta^{15}N$ or $\delta^{13}C$ and R is the corresponding ratio of $^{15}N/^{14}N$ or $^{13}C/^{12}C$, respectively.

Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope analyses were performed at the Environmental Quality Analysis Laboratory (Department of Biology, University of Regina, Regina, Saskatchewan). Dried samples of 1–2 mg were combusted using a NC2500 elemental analyzer (Thermoquest, CE Instruments) and introduced into a Finnigan MAT DeltaPlus mass spectrometer for gas isotope ratio measurements of $^{13}C/^{12}C$ and $^{15}N/^{14}N$. Standard materials were PeeDee belemnite limestone for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$. Two replicates of each sample were measured. Experimental precision, based on the standard deviation of replicates of the internal standard, was 0.38‰ for $\delta^{15}N$ and 0.08‰ for $\delta^{13}C$. Insufficient material was available to perform time series analysis of stable isotopes. Therefore, autotrophs, CPOM, and FPOM samples and consumer samples (by family) were pooled to generate composite isotopic signals for each group within each lake type, providing estimates of variation across three acidified and three recovered lakes. Owing to the minimal variation between isotopic baseline signals (~1 C unit and ~1 N unit) in the benthic food webs of each of the recovered and stressed lakes, data were combined to produce two figures that provide a more integrative illustration of the carbon flow and trophic structure as well as a more powerful analysis (as a result of more organisms for some taxa). Aggregation of whole-invertebrate or tissue samples eliminated individual variation in isotopic signature (Post 2002). To avoid a bias towards heavily sampled groups, when more than two $\delta^{15}N$ or $\delta^{13}C$ measurements per taxon were available in a lake, mean isotopic values were reported. The inferred trophic status of each organism was based on the reported relatively consistent 3.4‰ fractionation of $\delta^{15}N$ between trophic levels (Kling et al. 1992).

Statistical analyses

All data were normalized using natural $\log_{10}$ transformation prior to statistical analyses to help stabilize variances and meet assumptions of homogeneity of variance. Multiple regression analyses using all environmental variables were conducted to identify significant, independent predictors of consumer and autotrophic biomass (Quinn and Keough 2002). Stepwise multiple regressions with backward selection were performed using SYTSTAT version 10 (SPSS 2000). Repeated-measures analyses of variance (RM-ANOVAs) were used to evaluate the effects of chemical stress (i.e., acidity) on autotrophic and consumer biomass over the survey period (Scheiner and Gurevitch 2001). Data were checked for sphericity and if the assumption was violated, the P value was adjusted using the more conservative Greenhouse–Giesser method. Repeated-measures multivariate analyses of variance (RM-MANOVAs) were used to test for differences in the functional composition of autotrophic and benthic consumer communities between stressed and recovered lakes. These analyses were completed

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Table 3. Environmental predictors of benthic autotroph and consumer biomass based on multiple regression analysis of abiotic and biotic factors from six survey lakes with significant predictors in bold type.

	Autotroph biomass			Consumer biomass		
Effect	Coefficient	*F*	*P*	Coefficient	*F*	*P*
pH	**0.88**	**10.62**	**0.007**		0.21	0.66
log conductivity		0.98	0.35		0.32	0.59
log TN		1.30	0.28		0.99	0.34
log TDP	**–0.61**	**5.44**	**0.04**	**–0.85**	**9.94**	**0.007**
log DIC	**–3.64**	**6.17**	**0.03**		4.58	0.052
log DOC		0.46	0.51		0.07	0.8
log autotroph biomass		na	na		0.82	0.38
log consumer biomass		0.16	0.7		na	na
log grazer biomass		0.59	0.46		na	na

Note: na, not applicable.

using the general linear model procedure in SYSTAT version 10 (SPSS 2000). Variance ratios (VRs), based on the variance of total consumer biomass related to the cumulative variance of the component functional group biomass, were used to quantify compensatory dynamics for consumer communities (Frost et al. 1995; Vinebrooke et al. 2003*b*). The variance of total biomass is equal to the sum of component taxa biomass plus their covariances. Compensatory dynamics existed when the VR was less than 1, signifying negative covariance between the taxa. Otherwise, if VRs were equal to or greater than 1, then taxa varied independently or synchronously, respectively (Vinebrooke et al. 2003*b*). All VR testing was done on nontransformed data (Frost et al. 1995).

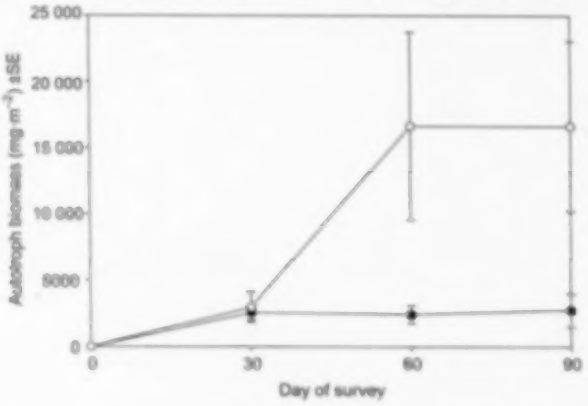
Canonical correspondence analysis (CCA) (CANOCO 4.51; ter Braak and Smilauer 2003) was used to examine relationships among environmental variables and the taxonomic composition of both autotrophic and consumer communities. Preliminary CA of taxonomic variation within both communities revealed that the lakes span a sufficient environmental (i.e., length of axis I >3 SD units) gradient to justify the use of a unimodal response model and CCA (ter Braak and Prentice 1988). The log transformations were done on all variables, including autotroph biovolume, consumer biomass, and water chemistry, with the exception of pH. This reduced the influence of large algal and consumer species and outliers that otherwise skew ordinations (ter Braak 1986). Automatic forward selection testing was used to screen the environmental data for redundant, and nonsignificant variables (ter Braak 1987). Biplot scaling was used to show the regression relations in CCA between species and species-derived sample scores, and Monte Carlo permutation tests were applied (499 iterations) to establish the significance of eigenvalues. Species that did not occur in at least three samples were omitted from ordination analyses owing to lack of variance.

Results

Lake water chemistry

The surveyed lakes varied (mean ± SD) in pH (5.9 ± 0.7), conductivity (25.9 ± 7.7 $\mu m \cdot s^{-1}$), TN (277.8 ± 47.4 $\mu g \cdot L^{-1}$), TDP (2.4 ± 1.6 $\mu g \cdot L^{-1}$), DOC (3.0 ± 1.7 $mg \cdot L^{-1}$), and DIC (1.0 ± 0.8 $mg \cdot L^{-1}$). Chemical analyses confirmed that the six survey lakes could be grouped into stressed and recovered categories (Table 1). Acid-stressed lakes had an average pH of 5.4 that remained below the biologically critical threshold of pH 6 (Schindler 1988). Recovered lakes had a less acidic average pH of 6.4, along with higher concentrations of DIC, DOC, and TDP. All lakes were oligotrophic, with TDP consistently higher in recovered lakes, especially in Little Sheguiandah Lake in May and June. TN and conductivity were comparable across study lakes during the survey.

Fig. 1. Mean total benthic biomass in stressed (solid circles) and recovering (open circles) lakes over the 90-day survey period (n = 3).



Autotrophic community structure

Differences in total algal biomass between stressed and recovered lakes were significant (stress, $F_{[1,4]} = 10.47$, $P = 0.03$) during summer 2003. Total biomass increased substantially in recovered lakes relative to that in stressed lakes following day 30 (Fig. 1). DIC, pH, and TDP were significant predictors of algal biomass, explaining 45.5% of the variation ($F_{[3,13]} = 3.62$, $P = 0.04$) (Table 3).

Gross taxonomic composition of autotrophs differed significantly between stressed and recovered lake (RM-MANOVA: time × stress, $F_{[14,56]} = 3.57$, $P < 0.001$) (Fig. 2). Filamentous green algae (FGA) made up 78% of autotrophic biomass in stressed lakes and 87% in recovered lakes with samples often being dominated by a single genus (e.g., *Mougeotia*, *Spirogyra*). Diatoms and desmids (RM-ANOVA: time × stress, desmids, $F_{[2,8]} = 3.54$, $P = 0.08$; diatoms,

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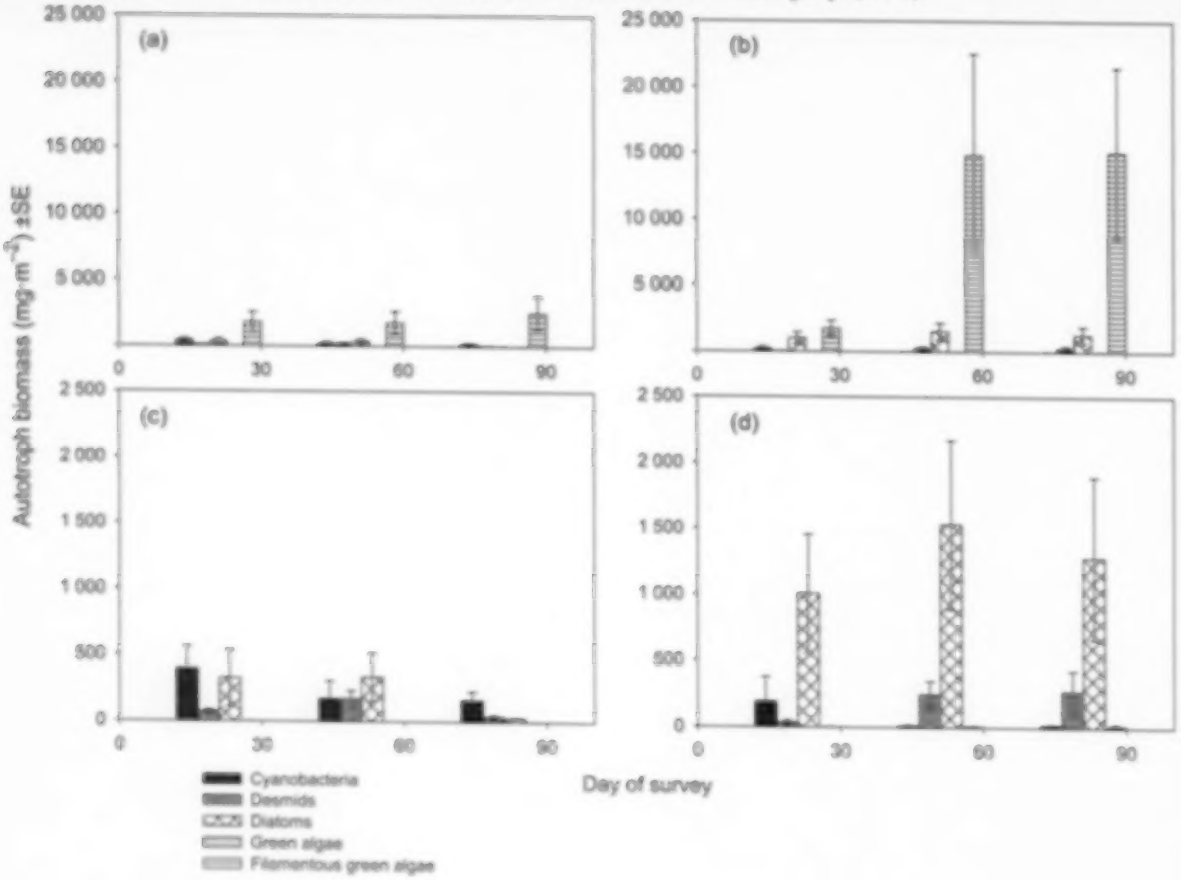
Fig. 2. Benthic autotroph biomass based on taxonomic group composition for stressed and recovering lakes. (*a* and *b*) All five taxonomic groups; (*c* and *d*) filamentous green algae omitted to show trends of less dominant groups (n = 3).



$F_{[2,8]}$ = 12.14, P = 0.004) were more abundant in recovered lakes. Although not statistically significant (RM-ANOVA: stress, $F_{[1,4]}$ = 5.42, P = 0.08), a higher proportion of cyanobacteria were found in stressed lakes.

CCA captured 54.4% of the total variance in species data and for 89.8% of the joint variance between algal and environmental variables and was significant (P < 0.01) (Fig. 3; Table 4). Thirty-three taxa were omitted because they occurred infrequently and did not influence the final ordination. The first axis (λ_1 = 0.36) was best described by DOC (r = –0.92) and pH (r = -0.69) and explained 28.0% of the species variance. CCA axis 1 separated *Homoeothrix* (No. 3), a clearwater (i.e., low DOC) species, from desmids such as *Euastrum* (No. 10) and *Gonatozygon* (No. 11) (Fig. 3*a*). In addition, the gradient contrasted acid-tolerant taxa (e.g., *Homoeothrix* (No. 3)) with acid-sensitive taxa (e.g., *Achnanthes* (No. 14) and *Gomphonema* (No. 18)). Site scores distinctively separate acid lakes from recovered lakes according to pH (Fig. 3*b*).

The second CCA axis (λ_2 = 0.15) explained 11.6% of the species variance and was positively correlated with DIC (r = 0.40). Site scores suggested that DIC increased in recovered lakes during the survey period, while DIC became less available for autotrophs in stressed lakes (Fig. 3*b*). The second axis contrasted certain diatoms, *Fragilaria* (No. 17) and *Pinnularia* (No. 21), with FGA (*Zygogonium* (No. 29)) and desmids (e.g., *Netrium* (No. 12) and *Gonatozygon* (No. 11)). Grazer biomass was retained as an independent significant variable following forward selection, explaining 3% of the total species variance. Forward selection testing revealed that time of sampling (i.e., seasonality) was not a significant explanatory variable.

Consumer community structure

TDP was the only significant predictor of consumer biomass, explaining 42.6% of the variation ($F_{[2,14]}$ = 5.19, P = 0.02) (Table 3). Total consumer biomass did not differ significantly between stressed and recovered lakes (RM-ANOVA: time × stress, $F_{[3,12]}$ = 0.61, P = 0.62) (Fig. 4*a*). Consumer biomass (R^2 = 0.02, P = 0.55) and grazer biomass (R^2 = 0.05, P = 0.77) were not significantly correlated with autotrophic biomass across all sampling dates and lakes (Fig. 5). If values were averaged monthly across lakes within each group, then correlations between autotrophic biomass

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Table 4. List of benthic algal taxa used in CCA.

Number	Taxon	Algal group
1	*Anabaena*	C
2	*Chroococcus*	C
3	*Homoeothrix*	C
4	*Lyngbya*	C
5	*Merismopedia*	C
6	*Oscillatoria*	C
7	*Phormidium*	C
8	*Bambusina*	DE
9	*Cylindrocystis*	DE
10	*Euastrum*	DE
11	*Gonatozygon*	DE
12	*Netrium*	DE
13	*Staurastrum*	DE
14	*Achnanthes*	DI
15	*Cymbella*	DI
16	*Eunotia*	DI
17	*Fragilaria*	DI
18	*Gomphonema*	DI
19	*Navicula*	DI
20	*Nedium*	DI
21	*Pinnularia*	DI
22	*Stenopterobia*	DI
23	*Tabellaria*	DI
24	*Bulbochaete*	FGA
25	*Mougeotia*	FGA
26	*Oedogonium*	FGA
27	*Spirogyra*	FGA
28	*Stigeoclonium*	FGA
29	*Zygogonium*	FGA
30	*Scenedesmus*	GA

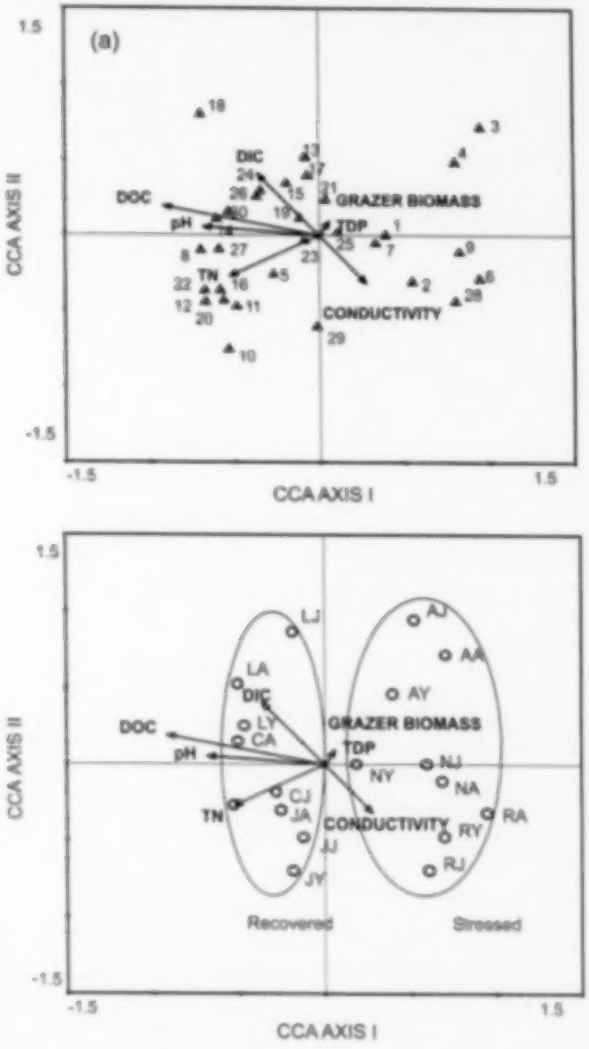
Note: Taxon identification numbers correspond to those used in the CCA plots. Autotrophs are grouped into cyanobacteria (C), desmids (DE), diatoms (DI), filamentous green algae (FGA), and green algae (GA).

and mean total consumer biomass (R^2 = 0.18, P = 0.40) or grazer biomass (R^2 = 0.13, P = 0.49) were also nonsignificant.

Differences in the functional composition of consumer communities between stressed and recovered lakes were marginally significant (RM-MANOVA: time × stress, $F_{[10,40]}$ = 1.91, P = 0.07) (Table 2; Fig. 4). Specifically, grazers were more abundant in recovered lakes (Fig. 4*b*), and omnivores were more common in stressed lakes (Fig. 4*c*), while predators did not differ consistently between lakes (Fig. 4*d*). Consumer groups exhibited negative covariances, resulting in VRs equaling less than 1 on day 30 (VR = 0.47), day 60 (VR = 0.45), and day 90 (VR = 0.44).

A greater proportion of total consumer biomass consisted of large-bodied consumers in recovered relative to stressed lakes (Fig. 6*a*). In particular, large consumers (>10 mm maximum body width) were present only in the recovered lakes. Small-bodied consumers were more common in stressed lakes, with the majority of consumers less than 2.5 mm wide (Fig. 6*a*). A shift from small to large grazers was evident from stressed to recovered lakes (Fig. 6*b*). A similar pattern was evident for omnivores (Fig. 6*c*). In both

Fig. 3. Association of (*a*) benthic autotrophs and (*b*) lake sampling sites with environmental variables across six survey lakes based on log-transformed taxa biovolumes, water chemistry, and grazer biomass data. Two-letter codes in Fig. 3*b* indicate lake (A, Acid; N, Norway; R, Ruth Roy; J, A.Y. Jackson; C, Carlyle; L, Little Sheguiandah) followed by date of sampling (J, June (day 30); Y, July (day 60); A, August (day 90)).



lake types, predators made the greatest contribution to total biomass in the 2.5–10 mm size class (Fig. 6*d*).

The taxonomic composition of consumer communities differed between stressed and recovered lakes. Of the 23 observed families, seven were absent from the stressed lakes and two were missing from the recovered lakes (Table 2). Corixids, chironomids, and odonates characterized consumer communities in stressed lakes and ephemeropterans, tadpoles, and minnows in recovered lake communities.

CCA captured 34.5% of the total variance in consumer taxonomic data with four axes. The first axis significantly

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Fig. 4. Biomass of (*a*) total benthic consumers, (*b*) grazers, (*c*) omnivores, and (*d*) predators in stressed lakes (solid circles) and recovering lakes (open circles) over the 90-day survey period ($n = 3$).

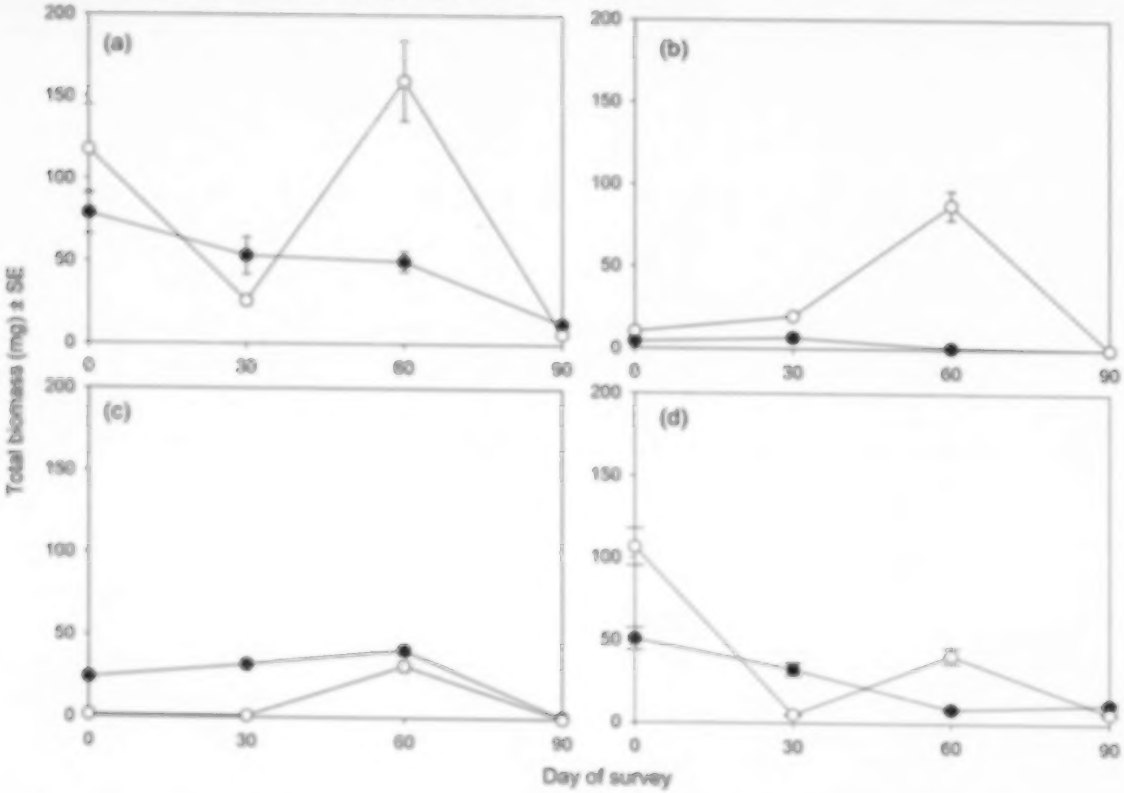
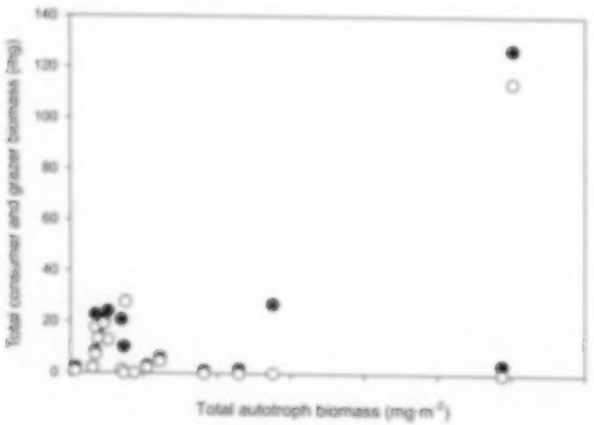


Fig. 5. Relationship between benthic consumer (solid circles) and grazer (open circles) biomass and autotrophic biomass across survey lakes ($n = 6$) during summer 2003.



($F = 2.31$, $P = 0.05$) explained 15.5% of the taxonomic variance and was best described by DOC ($r = 0.96$) and pH ($r = 0.81$) (Fig. 7). In particular, ranids (i.e., tadpoles (No. 3)) and cyprinids (No. 7) and to a lesser degree viviparids (No. 11), gammarids (No. 2), and ephemeropterans (No. 10) were more abundant in colored (i.e., higher DOC) circumneutral lakes. In contrast, corixids (No. 12), gyrinids (No. 6), and an small unidentifiable trichopteran were more dominant in more acidic, clear lakes.

CCA axis II was best correlated with autotrophic biomass ($r = -0.54$) and conductivity ($r = 0.62$) (Fig. 7). Herbivorous ranids (No. 3) and cyprinids (No. 7) were positively associated with the autotroph biomass vector. Gammarids (No. 2) and ephemeropterans (No. 10) inhabiting more chemically concentrated circumneutral lakes were separated along the conductivity axis from corixids (No. 12) in more acidic, dilute lakes. Time was identified as a nonsignificant explanatory variable based on forward selection testing.

Stable isotope signatures

The relative importance of benthic algae and detritus to consumers could not be inferred using stable isotopic data because $\delta^{13}C$ signals of base carbon sources were very similar (Fig. 8). Most benthic consumers had $\delta^{13}C$ values that were closely aligned with periphytic and detrital (CPOM, FPOM) signals. Certain consumers, including chironomids, dytiscids, gomphids, and notonectids in stressed lakes and gomphids, odonates, and asellids in recovered lakes, had more negative $\delta^{13}C$ signals derived potentially in part from unmeasured planktonic sources of carbon (i.e., depositional feeders or planktivores).

$\delta^{15}N$-inferred trophic positioning of benthic consumers, based on an approximate 3.4‰ fractionation of $\delta^{15}N$ be-

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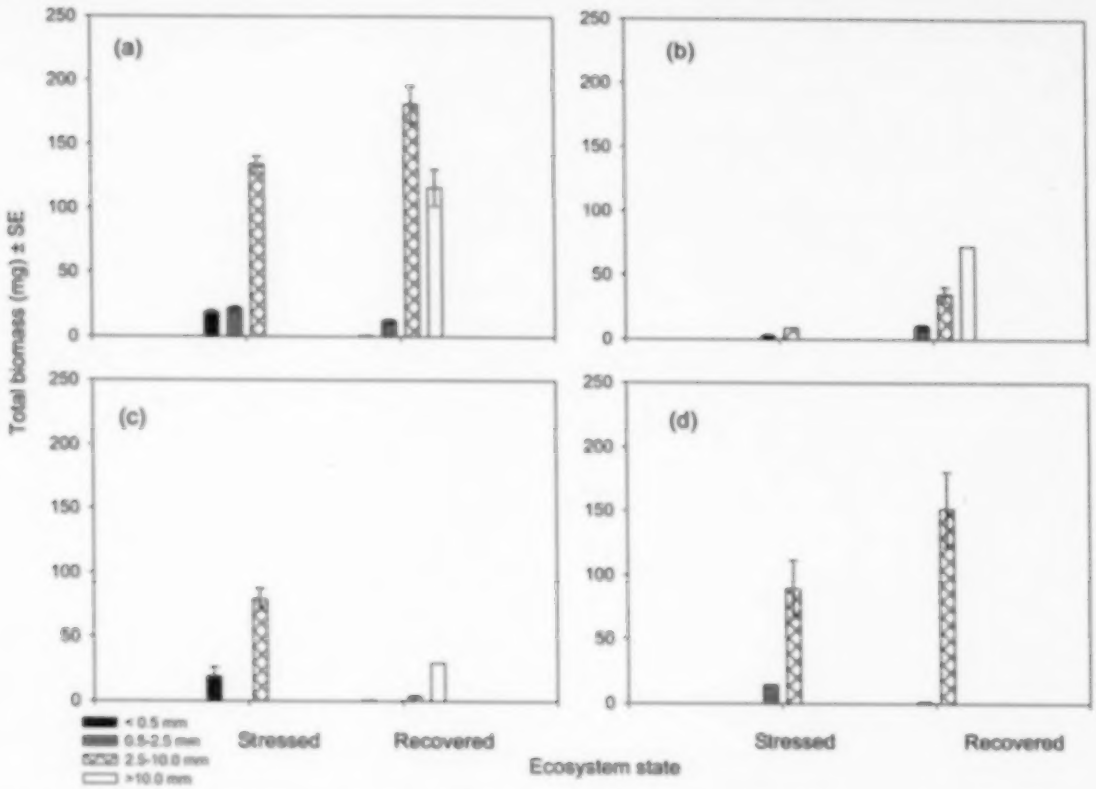
Fig. 6. Size structure of (*a*) total benthic consumer, (*b*) grazer, (*c*) omnivore, and (*d*) predator biomass in stressed and recovering lakes ($n = 3$).



tween trophic levels, revealed a greater diversity of primary consumers (e.g., grazers) in recovered lakes and secondary consumers (carnivores) in stressed lakes (Fig. 8). In stressed lakes, $\delta^{15}N$ values suggested that only Asellidae and Chironomidae functioned as primary consumers, while more consumers were positioned more than a full level (~3.4 $\delta^{15}N$ units) above that of detritus and periphyton (Fig. 8*a*). In contrast, six consumer families in the recovering lakes were positioned within one inferred trophic level of primary benthic carbon sources (Fig. 8*b*). Specifically, asellids, hyallids, limnephilids, viviparids, corydalids, and cambarids functioned as primary consumers of periphyton, CPOM, and FPOM (Fig. 8*b*).

Discussion

Resources or "bottom-up" factors were the primary predictors of benthic consumer and autotrophic community structure along a gradient consisting of six recovering acidified lakes. Greater nutrient availability and reduced acidity explained both increased autotrophic biomass and taxonomic shifts in consumer and autotrophic communities. Total consumer biomass did not differ between stressed and recovered lakes because of a compensatory size shift involving numerous, small acid-tolerant omnivores (asellids and chironomids) and fewer, large acid-sensitive omnivores (cyprinid minnows) and grazers (tadpoles). Further, consumers and autotrophic biomass were not significantly correlated and explained little variance in taxonomic composition, suggesting a weak trophic interaction (i.e., grazing) between these two communities. Below, we consider potential explanations for the observed relative importance of abiotic and biotic regulation of autotrophic and consumer community structure in acidified and recovering lakes.

Abiotic regulation of autotrophs

Abiotic regulation of benthic autotrophs in the Killarney lakes is consistent with findings from previous surveys of acid-stressed lakes (Turner et al. 1991; Vinebrooke 1996). Increased autotrophic biomass in the recovering lakes likely resulted from greater DIC availability and reduced carbon limitation under less acidic (pH > 6) conditions (Turner et al. 1991). In addition, a positive relationship between autotrophic biomass and DOC suggested that it functioned as a photoprotectant against DNA-damaging ultraviolet radiation (Vinebrooke and Leavitt 1996), a chelator of toxic metals (Kullberg et al. 1993), and a source of carbon to heterotrophic algae (Tuchman 1996). Greater autotrophic biomass in the recovering lakes was also positively related to higher TDP concentrations, but this relationship is likely unrelated to chemical recovery because acidification does not affect TDP levels in other boreal lakes (e.g., Vinebrooke et al. 2003*a*). If the recovered lakes contained inherently greater nutrient availability and primary production, then they may have been less affected by acidification because

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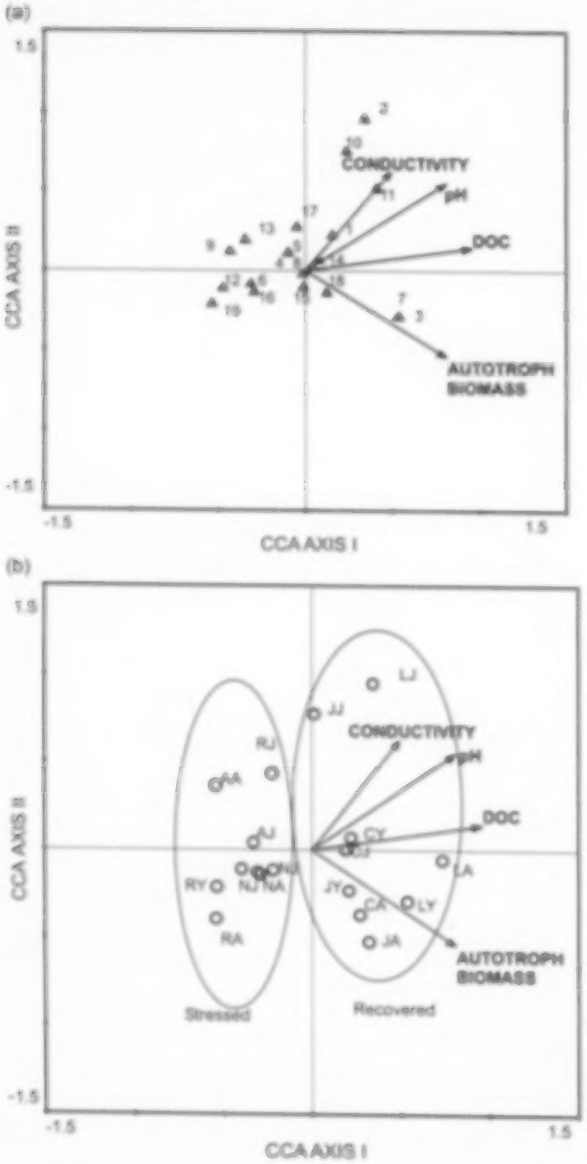
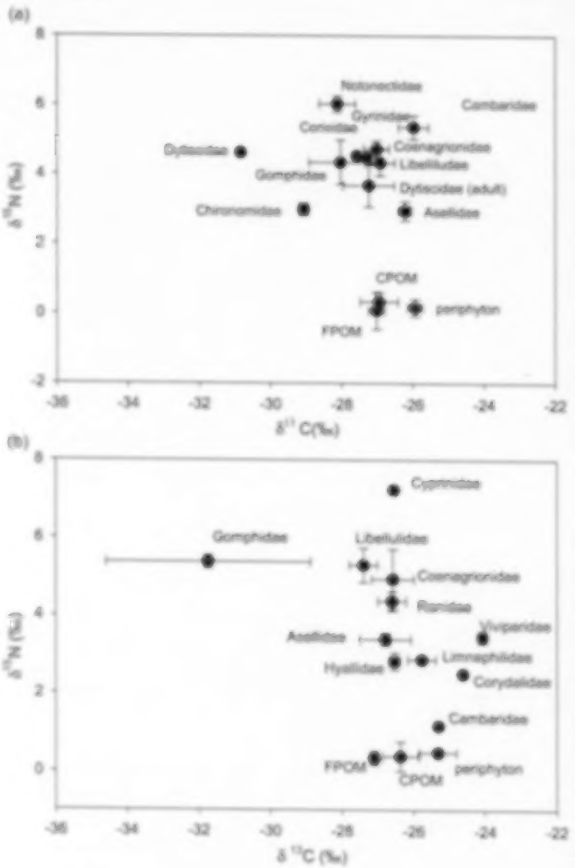
Fig. 7. Association of (*a*) benthic consumer taxa and (*b*) sampling site scores with environmental variables based on log-transformed consumer biomass, water chemistry, and autotrophic biomass data. Trichopterans (No. 19) that could not be identified to family are shown separately from members of the Limnephilidae family (No. 18). Two-letter codes in Fig. 3*b* indicate lake (A, Acid; N, Norway; R, Ruth Roy; J, A.Y. Jackson; C, Carlyle; L, Little Sheguiandah) followed by date of sampling (J, June (day 30); Y, July (day 60); A, August (day 90)).



primary producers generate base ions through the uptake and reduction of nitrate (Davison et al. 1995).

Autotrophic communities in both stressed and recovering lakes were dominated by FGA, which typically occur as midsummer blooms in both atmospherically and experimentally acidified lakes (e.g., Schindler et al. 1985; Turner et al. 1991). FGA, such as *Mougeotia* and *Zygogonium*, are known to proliferate in the littoral zone of acid-stressed lakes because theses zygnemataceans are superior competitors for inorganic carbon when DIC concentrations are low and grazing pressure is weak under acidic conditions (Turner et al. 1987; Graham and Vinebrooke 1998). However, more firmly attached forms of oedogoniacean FGA can also proliferate under circumneutral conditions (Graham and Vinebrooke 1998).

Fig. 8. Mean ± SE carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) values of periphyton, CPOM, FPOM, and benthic consumers collected from (*a*) stressed lakes and (*b*) recovering lakes ($n = 3$) during summer 2003.



Biotic regulation of autotrophs

The inferred effects of benthic grazers on autotrophs were subtle. Although autotrophic biomass was not a significant function of grazer biomass, we did detect a taxonomic shift from loosely attached, grazing-sensitive FGA (*Mougeotia* and *Zygogonium*) towards firmly attached, grazer-resistant FGA (*Oedogonium* and *Bulbochaete*) along the recovery gradient. Evidence from previous experiments also showed that benthic grazers often had stronger effects on taxonomic composition than on total biomass (Vinebrooke 1996; Graham and Vinebrooke 1998). The weak effect of benthic graz-

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ers on the total autotrophic biomass, consisting primarily of FGA, is likely due to large consumers (e.g., algivorous minnows) not heavily exploiting these algae as their primary carbon source (Vinebrooke et al. 2001). Further, morphological constraints of the mouthparts of small herbivorous invertebrates could release larger FGA from grazing pressure, enabling them to accrue large biomass in all of our study lakes. The general lack of a grazing effect might also reflect how consumer regulation is weak under oligotrophic conditions owing to herbivore populations being too sparse to exert a top-down effect on autotrophs (Elser and Goldman 1991).

Abiotic regulation of consumers

Our survey showed that pH and DOC best explained patterns in consumer community structure. Specifically, cyprinids, tadpoles, gammarids, and viviparids were restricted to the recovering circumneutral lakes with elevated DOC concentrations, while notonectids, corixids, and odonates were abundant in the severely acidified lakes. These findings support other studies that show distinct communities of acid-sensitive or acid-tolerant species depending on lake acidity (Schindler et al. 1985; Harvey and McArdle 1986; Carbone et al. 1998). For example, a pH of 6 has been identified as a critical threshold for many organisms, below which water conditions can become toxic (Schindler 1988; Havens et al. 1993; Holt and Yan 2003). Therefore, the primacy of abiotic factors structuring the consumer community was likely a result of our survey having been centered at about a pH of 6. Further, it is possible that aerial dispersal by many species (Keller et al. 1992; Bohonak and Jenkins 2003) has helped maintain a strong concordance between benthic invertebrate assemblages and recent improvements in water quality.

An increase in consumer size was a general response to chemical recovery in our survey lakes. The community shifted towards larger-bodied consumers with the reestablishment of acid-sensitive grazers in recovering lakes, owing possibly to suitable chemical conditions for growth and development. Similarly, decreased stress tolerance or higher energetic growth costs may explain the absence of larger consumers in acidified (Bilyj and Davies 1989) and other chemically polluted lakes (Cattaneo et al. 1998).

Biotic regulation of consumers

The relative minor importance of autotrophic biomass to benthic consumers agreed with the expected prevalence of omnivory and pelagic-littoral habitat coupling in boreal lakes (France 1998; Vinebrooke et al. 2001). The typical 3‰–4‰ enrichment in $^{15}\delta N$ per trophic level was not observed between most consumers and primary carbon sources, which is indicative of omnivorous diets (Kling et al. 1992). In addition, several consumers had highly variable $^{13}\delta C$ values, suggesting broad feeding habits that span different trophic levels from both benthic and pelagic habitats (Vander Zanden and Vadeboncoeur 2002; Herwig et al. 2004). Omnivory is hypothesized to function as an adaptative strategy, as consumers may have to feed at trophic levels with available energy and biomass despite traditional feeding habits, particularly in stressed habitats (Vadas 1990). In fact, our stable isotope analyses agree with the findings by Mihuc (1997) and Dangles (2002) that showed that many benthic invertebrates have far more flexible omnivorous diets under stressful conditions than indicated by traditional assignment to functional-feeding groups based on mouthpart morphologies.

Use of multiple carbon sources by zoobenthos is a potential explanation for the observed lack of a relationship between consumers and benthic autotrophs. Stable isotopic analyses suggested that autotrophs were not the only primary carbon source for the benthic food webs. Specifically, $^{13}\delta C$ signatures for most consumers were closely associated with both detritus (CPOM, FPOM) and autotrophic material. Vinebrooke et al. (2001) also concluded that detrital-based food webs are likely common in the littoral zone of boreal lakes based on stable isotopic analyses. In our study, omnivorous tadpoles and cyprinid minnows were the only organisms that relied heavily on autotrophic food based on ordination analyses. The importance of planktonic carbon sources to many consumers was also apparent in both lake groups.

Although visually feeding fish can structure littoral food webs (Bendell and McNicol 1995), the greater abundance of large potential prey taxa in the recovered lakes suggested that sparse populations of benthivorous fish did not strongly suppress zoobenthos. In acidified lakes, acid-tolerant predatory invertebrates are expected to strongly suppress other invertebrates (e.g., Appelberg et al. (1993), thereby possibly contributing to the observed size shift towards smaller grazers in the stressed lakes. The highest trophic levels in the stressed lakes were occupied by notonectids, which are known to increase abundance, along with corixids and odonates in other fishless lakes (Bendell and McNicol 1995). Therefore, we expect that a size shift in from small, tolerant predatory invertebrates to sensitive fish species led to trophic compensation (e.g., Elser et al. 1995), maintaining the magnitude of benthivory along the surveyed recovery gradient.

Compensatory species dynamics and ecosystem recovery

Our findings from a revisit of Killarney lakes suggested that abiotic factors remained the primary factors regulating benthic communities despite a decade of recovery having past since similar investigations were conducted (e.g., Vinebrooke 1996; Vinebrooke and Graham 1997). The importance of abiotic factors suggested that biological recovery was incomplete in these lakes. Alternatively, compensatory taxonomic shifts in benthic consumer communities may maintain the magnitude of herbivory during ecosystem recovery, thereby negating differences in grazing pressure between chemically stressed and recovered lakes. However, taxonomic shifts towards larger grazers appeared to affect feeding selectivity and therefore altered autotrophic community composition. Therefore, the role of benthic grazing as an ecological mechanism regulating recovery in acid-stressed lakes still warrants further study.

Acknowledgements

We thank the staff at Killarney Park for their support of our research. Mark Graham and Lindsay Hogsden provided invaluable assistance in the field. Funding for this research

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was provided by a Natural Sciences and Engineering Research Council of Canada Discovery Grant to R.D.V. We gratefully acknowledge financial support during manuscript preparation from the University of Alberta to K.L.H.

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Imbalance of fatty acids in the base of the Baltic Sea food web — a mesocosm study

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Abstract: A reproductive disturbance in Baltic Sea Atlantic salmon (*Salmo salar*), the M74 syndrome, has been reported since early 1970s and has occasionally caused up to 90% mortality for newborn fry. Previous research has revealed that the M74 syndrome may be due to reduced levels of the vitamin thiamin, the carotenoid astaxanthin, and elevated ratios of ω3/ω6 fatty acids in salmon eggs. Using mesocosm experiments, we compared the quantity (μg·L^{-1}) and quality (mg·g^{-1} C) of fatty acids in microalgae and copepods in the southern Baltic Sea where the M74 syndrome is common with those in a habitat in the Norwegian Sea where the syndrome has not been observed. Daily additions were made of the nutrients N and P or N, P, and Si, copepods were added after 6–7 days, and nutrient additions were stopped after 9–10 days. Flagellates dominated completely in the Baltic Sea, whereas higher phytoplankton diversity was found in the Norwegian Sea. We found elevated ω3/ω6 ratios in phytoplankton and abnormally high docosahexaenoic acid/arachidonic acid ratios (22:6ω3/20:4ω6) in copepods in the Baltic Sea mesocosms compared with those in the Norwegian Sea. Our results suggest that imbalance in fatty acid composition may prevail in the basic food web of the Baltic Sea.

Résumé : On a signalé depuis le début des années 1970 une perturbation de la reproduction, le syndrome M74, chez le saumon atlantique (*Salmo salar*) de la Baltique qui, à l'occasion, cause une mortalité pouvant atteindre 90 % chez les alevins néonates. Les études antérieures ont montré que le syndrome M74 peut être dû à des concentrations faibles de la vitamine thiamine et du caroténoïde l'astaxanthine et à des rapports élevés des acides gras ω3/ω6 dans les oeufs de saumons. Dans des expériences de mésocosmes, nous avons comparé la quantité (μg·L^{-1}) et la qualité (mg·g^{-1} C) des acides gras dans les microalgues et les copépodes dans le sud de la Baltique où le syndrome M74 est répandu à celles d'un habitat de la mer de Norvège où le syndrome n'a pas été observé. Nous avons ajouté quotidiennement les nutriments N et P ou alors N, P et Si, introduit des copépodes au bout de 6–7 jours et arrêté les additions de nutriments après 9–10 jours. Dans la Baltique, les flagellés sont dominants, alors que, dans la mer de Norvège, le phytoplancton est plus diversifié. Dans les mésocosmes de la Baltique, les rapports ω3/ω6 du phytoplancton sont élevés et les rapports acide docosahexanoïque/acide arachidonique (22:6ω3/20:4ω6) anormalement grands chez les copépodes, par comparaison aux mésocosmes de la mer de Norvège. Nos résultats laissent croire qu'il peut y avoir un déséquilibre dans la composition en acides gras dans le réseau alimentaire de base de la Baltique.

[Traduit par la Rédaction]

Introduction

The brackish Baltic Sea (54°N–66°N) has been enriched by N and P since the 1960s, and as a consequence, Si has started to decrease in the southern Baltic Sea (Elmgren and Larsson 2001). As a result, the spring blooms of diatoms have gradually been replaced by dinoflagellates, other flagellates, and cyanobacteria (Wasmund et al. 1998). Diatom records in the sediments of the southern Baltic Sea show that the eutrophication process has been progressing for at least 100 years, and that it accelerated in the 1970s (Snoeijs 1998). A shift in zooplankton has also occurred, from larger taxa such as mysids and larger copepods to smaller taxa such as rotifers and cladocerans (Flinkman et al. 1998; Karlsson et al. 1999). The changes in the chemical composition of the Baltic Sea have probably influenced the whole food web, perhaps culminating in a reproductive disturbance in Baltic Sea populations of Atlantic salmon (*Salmo salar*), which was first observed in 1974 (Bengtsson et al. 1999). This disturbance, known as the M74 syndrome, varies

Received 17 May 2004. Accepted 27 May 2005. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on 16 September 2005.
J18139

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 doi: 10.1139/F05-140 © 2005 NRC Canada

greatly between years, and in some years, up to 90% of the female salmon produce offspring with this syndrome, characterized primarily by neurological symptoms (Bengtsson et al. 1999; Lundström et al. 1999). During 2001, when our study took place, 28% of salmon fry were affected. A similar disturbance, known as the early mortality syndrome, has been found in salmonids in the North American Great Lakes (Fitzsimons et al. 1999).

The food quality of phytoplankton has been associated with among other things certain biochemicals such as fatty acids (FAs), which can vary greatly between phytoplankton groups (Volkman et al. 1989; Ahlgren et al. 1992). Some FAs are also specific for different groups and can be used as markers for the transfer of organic matter through food chains (Léveillé et al. 1997; Goedkoop et al. 1998; Dalsgaard et al. 2003). In general, diatoms contain high levels of palmitoleic acid (PA) (16:1ω7) and moderate levels of eicosapentaenoic acid (EPA) (20:5ω3) and arachidonic acid (ARA) (20:4ω6). Flagellates (e.g., cryptomonads and dinoflagellates) contain high levels of both EPA and docosahexaenoic acid (DHA) (22:6ω3) but have no ARA. Green algae contain high levels of α-linolenic acid (ALA) (18:3ω3) and linoleic acid (LA) (18:2ω6), which can serve as precursors to EPA and ARA, respectively. Many cyanobacteria completely lack or only contain small amounts of ALA and (or) LA. Traces of EPA have been noticed in a few cyanobacteria species (Ahlgren et al. 1992). Two groups of polyunsaturated FAs (PUFAs), the so-called ω3 and ω6 FA types, are essential for all animals and the optimal ω3/ω6 ratio is probably more or less species specific but is not known for most species (Sargent et al. 1995). The three most important FA in vertebrates are EPA, DHA, and ARA, and all three are so-called biologically active eicosanoids that are vital components of cell membranes and have regulatory functions in living cells, particularly for reproduction (Crawford et al. 1989; Harrison 1990). DHA is an important substance in vascular and neural tissue, such as the central nervous system, retina, and olfactory nerve, whereas ARA is important in certain eicosanoids, which control many systems in the body (e.g., prostaglandin formation in vertebrates) (Sargent et al. 1997). It has been suggested that the seston content of these FAs strongly influences the efficiency of the trophic transfer in aquatic food webs (Fraser et al. 1989; Brett and Müller-Navarra 1997). These FAs also have importance for ecosystem health and stability and are highly conserved in aquatic food chains (Arts et al. 2001).

Many hypotheses about the causes of the M74 syndrome have been presented (e.g., anthropogenic pollution with organic toxic substances and heavy metals, genetic degeneration, and increased eutrophication owing to nutrient inputs) (Bengtsson et al. 1999). The ultimate cause of the dramatic reproductive disturbance in salmon is still unclear, but the symptoms have been connected with a diet-related thiamin deficiency (Amcoff et al. 1998*a*, 1998*b*), low astaxanthin levels (Pettersson and Lignell 1999), and elevated levels of ω3 FA in affected Baltic Sea salmon eggs (Pickova et al. 1998). Experimental studies on the antioxidative systems in salmon have revealed that peroxidation mechanisms are involved in the pathogenesis of M74, indicating oxidative stress (Lundström 2000). Several factors causing changes in the Baltic Sea food chains are probably involved (Bengtsson et al. 1999). Therefore, we focus on the lower part of the food web in the Baltic Sea. The aims of our studies were to compare the food quality, in terms of carotenoids, thiamin, and FAs, of phytoplankton and copepods in two different habitats, one situated in the southern part of the Baltic Sea where M74 is common and the other in the Norwegian Sea on the west coast of Norway where M74 is not found. We simulated increased eutrophication by adding nutrients to natural seawater in outdoor mesocosms to stimulate different phytoplankton blooms. In the present paper, the dynamics of FAs in seston (hereafter referred to as phytoplankton) and copepods will be presented, whereas those of carotenoids and thiamin are presented elsewhere (Andersson et al. 2003; Van Nieuwerburgh et al. 2004*a*, 2004*b*; Wänstrand 2004). The following questions are addressed. (*i*) Do FAs change in quantity (micrograms per litre) and quality (mg·g^{-1} C) with nutrient treatments in the mesocosms as time proceeds? (*ii*) Do FAs in the phytoplankton differ between mesocosms in the habitats with and without M74? (*iii*) Do the levels of the biologically active eicosanoids DHA and ARA differ in copepods from the habitat with the M74 syndrome and in the habitat where the syndrome is absent? (*iv*) Can phytoplankton FAs be traced in the copepods?

Methods

Two habitats were chosen for the mesocosm studies: the Norwegian Sea (Hopavågen, 63°40′N, 9°39′E, late August – early September 1999) with no M74, which served as a control site, and the southern Baltic Sea (Zingst, 54°27′N, 12°40′E, early September 2001) where M74 is prevalent. The design of the experiments has been described in detail by Van Nieuwerburgh et al. (2004*a*) for Hopavågen and by Van Nieuwerburgh (2004) for Zingst. Briefly, six 2000-L (Hopavågen) or 1400-L (Zingst) mesocosms were used, with triplicates for two different treatments: daily additions of N + P (NP treatment) or N + P + Si (NPSi treatment). The concentrations used in these nutrient additions were 4 µmol N·L^{-1}, 0.25 µmol P·L^{-1}, and 12 µmol Si·L^{-1} daily. Copepods, collected from the field, were added on days 6 and 7, respectively, in the two habitats and nutrient additions were stopped on days 9 and 10, respectively, to study the effects of a collapsing phytoplankton bloom. Daily samplings were performed for analyses of particulate organic carbon (POC), chlorophyll *a* (Chl), and FA composition in phytoplankton and copepods. Phytoplankton samples, separated from copepods by filtering through a 200-µm-mesh net, were collected on GF/F glass-fiber filters, whereas copepods were collected on GF/C glass-fiber filters. The filters with phytoplankton and copepod samples were freeze-dried and stored at –80 °C until analysis. The C concentration of phytoplankton and copepods was analyzed using an elemental analyzer (LECO CHNS-932) and phytoplankton Chl was extracted with methanol and determined spectrophotometrically according to the Norwegian Standard NS 4767 (cf. Van Nieuwerburgh 2004).

FAs were measured as their methyl esters using a gas chromatograph (Hewlett-Packard 5890 GC) according to a method described in detail by Boberg et al. (1985). A modification of the method was that the Folch extraction (methanol–chloroform) was performed on the glass-fiber fil-

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Table 1. *P* values for single-factor repeated-measures analyses of variance for phytoplankton and copepod samples from the mesocosms with time (experimental day) as repeated measurable variable and with habitat as additional variable.

Variable	Phytoplankton NP	Phytoplankton NPSi	Copepod NP	Copepod NPSi
Group 1				
C ($mg \cdot L^{-1}$)	**<0.001**	**0.001**	**0.021**	**0.016**
Chl ($\mu g \cdot L^{-1}$)	0.868	0.144		
Density ($individuals \cdot L^{-1}$)			0.081	0.022
Chl/C ($mg \cdot g^{-1}$)	0.329	0.878		
Size ($\mu g\ C \cdot individual^{-1}$)			**0.001**	**<0.001**
Group 2				
ΣSAFAs ($\mu g \cdot L^{-1}$)	**<0.001**	**<0.001**	0.84	0.549
ΣMUFAs ($\mu g \cdot L^{-1}$)	**<0.001**	**<0.001**	0.932	0.336
ΣPUFAs ($\mu g \cdot L^{-1}$)	**<0.001**	**<0.001**	0.351	0.7
Σω3 ($\mu g \cdot L^{-1}$)	**<0.001**	**<0.001**	0.348	0.847
Σω6 ($\mu g \cdot L^{-1}$)	**<0.001**	**<0.001**	0.621	0.024
PA ($\mu g \cdot L^{-1}$)	**<0.001**	**<0.001**	0.323	0.129
EPA ($\mu g \cdot L^{-1}$)	0.009	**<0.001**	0.067	0.907
DHA ($\mu g \cdot L^{-1}$)	0.138	0.012	0.622	0.950
ARA ($\mu g \cdot L^{-1}$)	—	—	0.788	0.054
Group 3				
ΣSAFA/C ($mg \cdot g^{-1}$)	**0.001**	**<0.001**	**<0.001**	**<0.001**
ΣMUFA/C ($mg \cdot g^{-1}$)	0.167	0.016	**<0.001**	**<0.001**
ΣPUFA/C ($mg \cdot g^{-1}$)	0.834	**0.003**	0.240	0.007
Σω3/C ($mg \cdot g^{-1}$)	0.176	0.545	0.361	0.010
Σω6/C ($mg \cdot g^{-1}$)	**0.001**	**<0.001**	0.534	<0.050
PA/C ($mg \cdot g^{-1}$)	**0.006**	**<0.001**	**<0.001**	**<0.001**
EPA/C ($mg \cdot g^{-1}$)	0.156	0.186	0.036	**0.002**
DHA/C ($mg \cdot g^{-1}$)	0.304	0.628	0.179	0.658
ARA/C ($mg \cdot g^{-1}$)	—	—	0.307	**0.005**
Group 1				
ΣPUFAs/ΣSAFAs	**<0.001**	0.061	**0.001**	**<0.001**
Σω3/Σω6	**<0.001**	**0.001**	0.074	0.653
DHA/ARA	—	—	0.180	**<0.001**

Note: For comparing the concentrations ($\mu g \cdot L^{-1}$) in the two habitats, the percent changes since day 1 of the experiments were used as input data because the initial phytoplankton and copepod biomasses were different. Days 13 and 14 of the southern Baltic Sea experiment were excluded for the phytoplankton data as well as the last day for the copepod data when comparing the habitats because no corresponding days existed for the Norwegian Sea experiment. To guarantee that the overall significant test is still at the $P < 0.05$ level (bold entries), we performed Bonferroni correction by dividing 0.05 by the number of tests within the three different groups below (i.e., 0.05/3 = 0.017, 0.05/8 = 0.0063, and 0.05/9 = 0.0056, respectively). —, not calculated because ARA concentrations were the below detection limit in most of the samples from the southern Baltic Sea.

ters with the samples instead of on preweighed samples. Individual FAs were identified by comparing their retention times with those of several commercially available standard mixtures (GCL-68A; NU CHEK Prep. Inc.) mixed with Fish oil 30 (Larodan Fine Chemicals AB). The FAs were quantified (micrograms per litre) by injection of fixed volumes of the extracts into the GC and comparing the area of the peaks with the peak of an internal standard, 0.25 or 0.10 mg·sample tricosanoic $acid^{-1}$ (23:0). The detection limit of this technique is about 0.02 mg·g dry $weight^{-1}$.

The variation was usually very small in the replicates of POC and Chl data (Van Nieuwerburgh et al. 2004*a*; Van Nieuwerburgh 2004). Therefore, and because of the high costs of analysis of FAs, only one of the triplicate mesocosms was randomly selected for FA analyses (mesocosms 2 and 6 at Hopavågen and 2 and 7 at Zingst). Differences between the two habitats were tested by a single-factor repeated-measures analysis of variance in which experimental units are recorded repeatedly through time. However, this means that the difference between the specific microcosms can be established but not that the treatments as a whole were different. Box-Cox transformations were used when the distribution of the data was not normal. Throughout this paper, $P < 0.05$ was used as the statistical significance level. To guarantee that the overall significant test is still at the 0.05 level, Bonferroni correction was performed by dividing 0.05 by the number of tests in the three different groups in Table 1 (i.e., 0.05/3 = 0.017, 0.05/8 = 0.0063, and 0.05/9 = 0.0056, respectively). In the regressions between phytoplankton and copepod FAs, $P \leq 0.1$ is also shown.

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Fig. 1. (*a–f*) Concentrations of C, Chl, and Chl/C ratio versus day for phytoplankton in the two mesocosm experiments; (*g–l*) concentrations of C, density, and size for copepods (Cop) in the two mesocosm experiments. Two mesocosms were placed in the Norwegian Sea where M74 is absent and two in the southern Baltic Sea where M74 is common. In each habitat, one of the mesocosms was treated with additions of NP (shaded circles) and the other with NPSi (solid circles) in phytoplankton. Arrows indicate the end of the nutrient additions. Broken lines, Chl/C = 20 mg·g^{-1} or 0.02 g·g^{-1}.

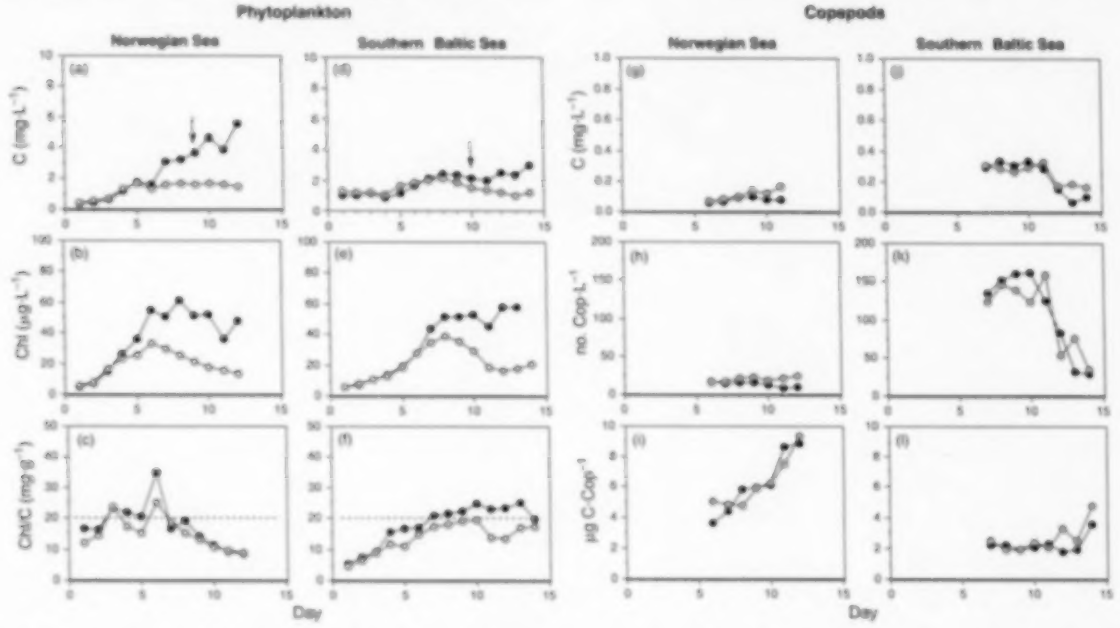

Table 2. Dominant groups of phytoplankton and genera of copepods in the Norwegian Sea and southern Baltic Sea in the different treatments at the beginning (control) and end of the mesocosm experiments.

Treatment	Norwegian Sea (Hopavågen)	Southern Baltic Sea (Zingst)
Algal groups		
Control	Prymnesiophytes + chrysophytes 40%	Flagellates 70%
	Dinoflagellates 30% (e.g., *Ceratium*)	Dinoflagellates 20%
	Diatoms 20% (e.g., *Chaetoceros*, *Cylindrotheca*/*Nitzschia*), others 10%	Diatoms 10%
NP	Prymnesiophytes + chrysophytes 50%	Flagellates 70% (of which 50% are cryptophytes)
	Dinoflagellates 40%	Dinoflagellates 20%
	Diatoms 5%, others 5%	Diatoms 5%, others 5%
NPSi	Diatoms (heavily silicified) 90% (mainly *Thalassiosira*)	Diatoms 80% (mainly centric diatoms)
	Prymnesiophytes + chrysophytes 5%	Flagellates 20%
	Dinoflagellates 5%	
Copepod genera		
Control	*Acartia* 34%, *Pseudo*/*Paracalanus* 24%	*Acartia* 90%,
	Temora 22%, *Oithona* 16%	*Eurytemora* 9%
	Centropages 3%, *Calanus* 0.2%	*Temora* 1%

Note: The taxonomic composition of the copepods did not change with time or treatment.

Results

Phytoplankton and copepod abundance and composition

The C concentration of phytoplankton (µg·L^{-1}) in the NPSi treatment increased with time with a steeper slope and showed significantly higher levels in the Norwegian Sea than in the southern Baltic Sea (Figs. 1*a* and 1*d*; Table 1). The NP treatments showed smaller but similar increases with time (Figs. 1*a* and 1*d*). Chl concentrations showed similar temporal patterns in both habitats; in the NPSi treatments, Chl concentrations reached a more or less steady level at 50–60 µg·L^{-1}, but in the NP treatments, they peaked at days 6 and 8 in the two habitats, respectively (Figs. 1*b* and 1*e*). The phytoplankton Chl/C ratio in both Norwegian Sea treatments fluctuated during the first 6 days, after which it decreased steadily. In contrast, in the southern Baltic Sea mesocoms, this ratio steadily

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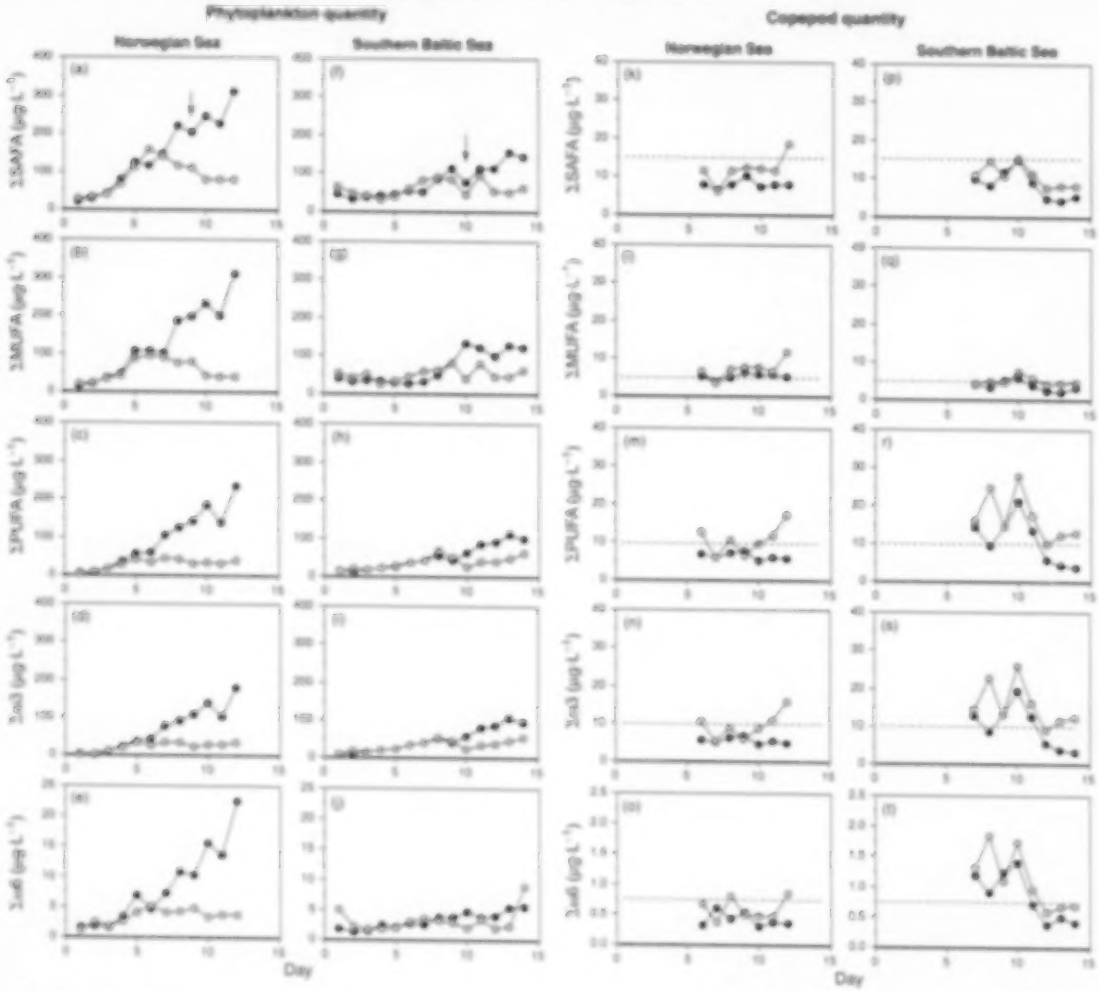
Fig. 2. Concentrations of ΣSAFAs, ΣMUFAs, ΣPUFAs, Σω3, and Σω6 versus day in the two mesocosm experiments in (*a–j*) phytoplankton and (*k–t*) copepods. Shaded and solid circles indicate NP and NPSi treatments, respectively. Arrows indicate the end of the nutrient additions. Broken lines make comparisons between the two habitats easier.



increased until day 10 when nutrient additions were stopped (Figs. 1*c* and 1*f*). Chl concentrations and Chl/C ratios were not significantly different between the two habitats (Table 1). On the contrary, the plankton flora was quite different in the two habitats and changed in the NPSi treatments during the experiments towards complete diatom dominance (Table 2). In the Norwegian Sea, high taxonomic diversity was observed with about the same occurrences of prymnesiophytes, chrysophytes, dinoflagellates, and diatoms in the NP mesocosm, whereas flagellates such as cryptophytes and dinoflagellates dominated in the southern Baltic Sea. In the NPSi mesocosm, larger diatoms belonging to the genus *Thalassiosira* dominated in the Norwegian Sea mesocosm, whereas smaller centric diatoms such as *Thalassiosira* spp. and *Skeletonema costatum* dominated in the southern Baltic Sea mesocosm.

The zooplankton fauna, which showed almost no compositional changes during the experiments, was also more diverse in the Norwegian Sea than in the southern Baltic Sea (Table 2). The large copepods *Pseudocalanus* and *Paracalanus*, which were common in the Norwegian Sea mesocosms, were absent in the southern Baltic Sea experiment. Instead, smaller *Acartia* species dominated in the southern Baltic Sea mesocosms. The copepods in the Norwegian Sea were larger than those in the southern Baltic Sea and grew both in number and in body size in the NP treatments but only in body size in the NPSi treatment (Figs. 1*g–1i*). Copepod density decreased dramatically in both treatments in the southern Baltic Sea and the C content per individual was low except for the last day (Figs. 1*j–1l*).

Quantitative aspects of FAs (μg·L⁻¹)

The quantity of the three groups of phytoplankton FAs in the NP mesocosm in the Norwegian Sea, the sum of saturated FAs (ΣSAFAs), monounsaturated FAs (ΣMUFAs), and PUFAs (ΣPUFAs) showed a steady increase from day 1 until days 5 or 6 and then leveled off (Figs. 2*a–2c*). (Concentra-

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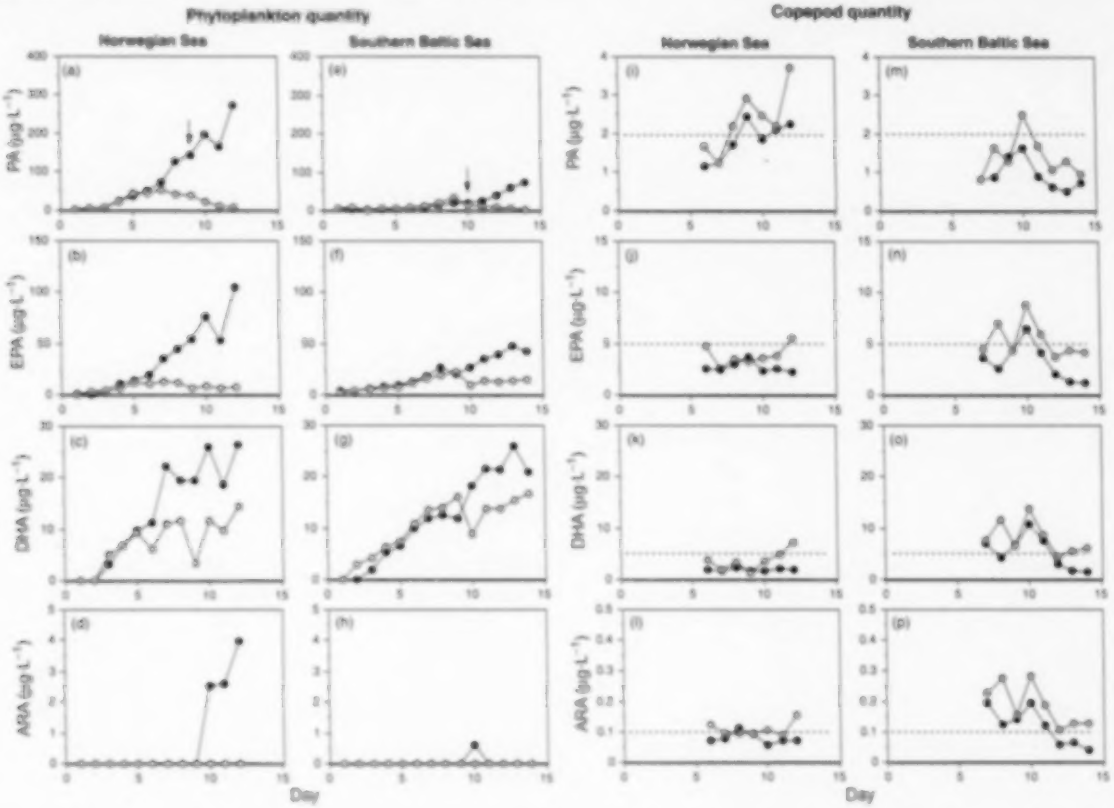
Fig. 3. Concentrations of PA, EPA, DHA, and ARA versus day in the two mesocosm experiments in (*a–h*) phytoplankton and (*i–p*) copepods. Shaded and solid circles indicate NP and NPSi treatments, respectively. Arrows indicate the end of the nutrient additions. Broken lines make comparisons between the two habitats easier.



tions of all FAs are available in supplementary appendices S1 (Norwegian Sea) and S2 (Baltic Sea) in the National Research Council Data Depository online.)[2] In the NPSi mesocosm, the increase continued throughout the whole 12-day experiment, even though the nutrient additions were stopped on day 9. In contrast, the development of the same three FA groups in both mesocosms of the southern Baltic Sea showed significantly lower levels, which leveled off after day 10 (Figs. 2*f*–2*h*). The two PUFA groups, Σω3 and Σω6, showed about the same pattern as ΣPUFAs in both habitats (Figs. 2*d*, 2*e*, 2*i*, and 2*j*). In both the NP and NPSi treatments, all FA groups including Σω3 and Σω6 PUFAs showed significant differences between the two habitats (Table 1). The diatom-indicating FA palmitoleic acid (PA) (16:1ω7) showed a steep positive slope and significantly higher concentrations in the Norwegian Sea than in the southern Baltic Sea (Figs. 3*a* and 3*e*). The two most important ω3 FAs, EPA and DHA, showed low concentrations in the NP mesocosm but rapidly increasing concentrations in the NPSi mesocosm in the Norwegian Sea (Figs. 3*b* and 3*c*). In the southern Baltic Sea, EPA was at relatively low concentrations, but DHA gradually increased to the same high levels as in the Norwegian Sea in the NPSi mesocosm and even to higher concentrations in the NP mesocosms (Figs. 3*f* and 3*g*). The most important ω6 PUFA, ARA, was present at low concentrations in the Norwegian Sea NPSi mesocosm during the last 3 days of the experiment but was only found in trace amounts on one occasion in the southern Baltic Sea (Figs. 3*d* and 3*h*).

The FA quantity (µg·L^{-1}) in the copepods was one order of magnitude lower than in the phytoplankton despite that the added copepods were concentrated (notice the different scales between phytoplankton and copepods in Figs. 1–3). ΣSAFAs went up to about 15 µg·L^{-1} and ΣMUFAs to about 8 µg·L^{-1} in both habitats, whereas ΣPUFAs showed high and variable concentrations in the southern Baltic Sea, up to 27 µg·L^{-1} (Figs. 2*k*–2*m* and 2*p*–2*r*). FAs of the Σω3 and Σω6 type were relatively low and stable in the Norwegian Sea but more variable in the southern Baltic Sea (Figs. 3*n*, 3*o*, 3*s*, and 3*t*). However, they decreased in the southern Baltic Sea with a steep slope after day 10 when the nutrient additions were stopped. PA (relative data) did not show

[2] Supplementary data for this article are available on the Web site or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Ottawa, ON K1A 0R6, Canada. DUD 4028. For more information on obtaining material refer to http://cisti-icist.nrc-cnrc.gc.ca/irm/unpub_e.shtml.

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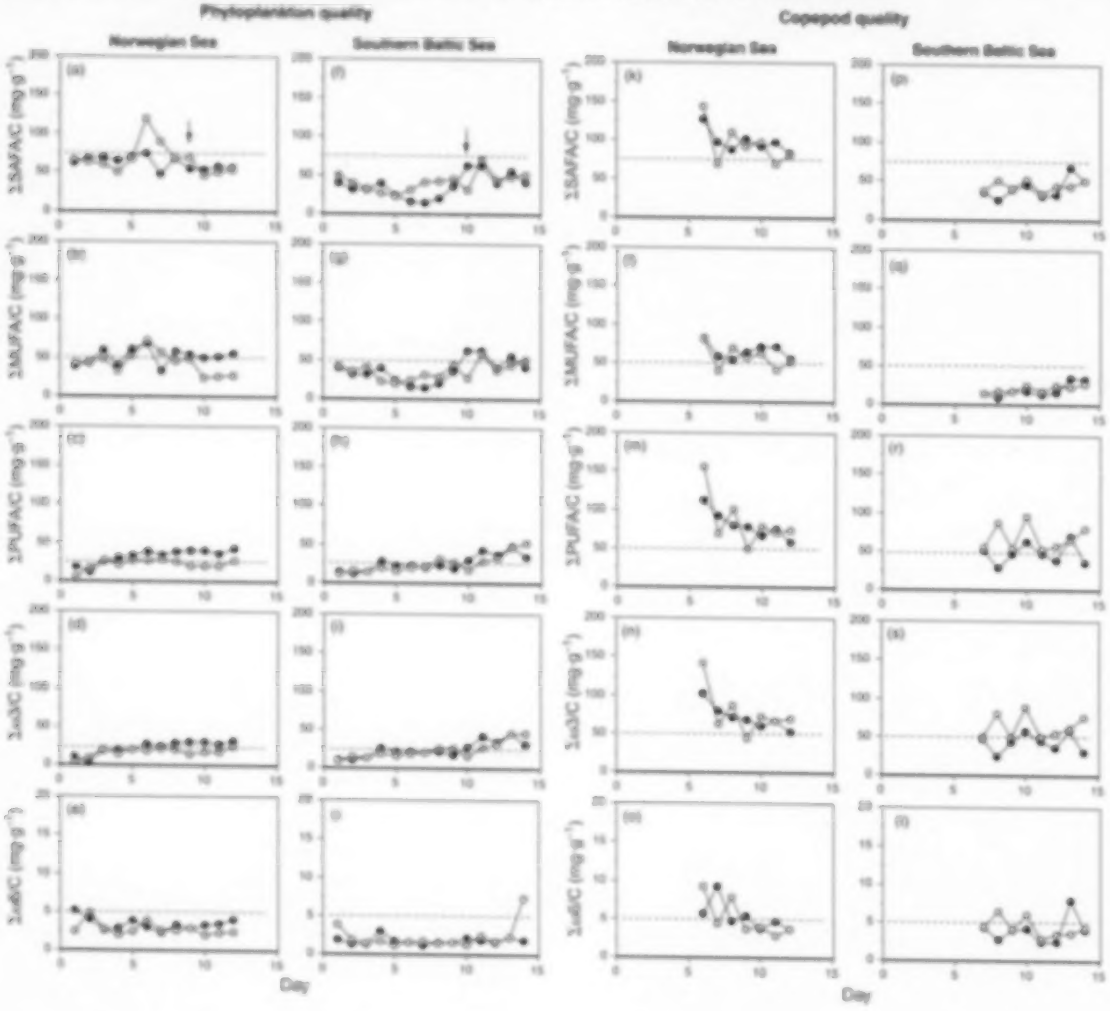
Fig. 4. Content of groups of FA per C: ΣSAFAs/C, ΣMUFAs/C, ΣPUFAs/C, Σω3/C, and Σω6/C versus day in the two mesocosm experiments in (*a–j*) phytoplankton and (*k–t*) copepods. Shaded and solid circles indicate NP and NPSi treatments, respectively. Arrows indicate the end of the nutrient additions. Broken lines make comparisons between the two habitats easier.



significant differences between the two habitats (Figs. 3*i* and 3*m*). In the NPSi treatment, copepod PA increased significantly with time in the Norwegian Sea with a rate of 0.19 $\mu g \cdot L^{-1} \cdot day^{-1}$ ($R^2 = 0.65$, $P = 0.03$), whereas EPA, DHA, and ARA were stable. Except for the last 3 days of the experiments, EPA, DHA, and ARA showed higher concentrations in the southern Baltic Sea than in the Norwegian Sea (Figs. 3*j–3l* and 3*n–3p*).

Qualitative aspects of FAs ($mg \cdot g^{-1}$ C)

The qualitative aspect of phytoplankton FAs showed another picture than the quantitative aspect. All FA groups were relatively stable with time in both nutrient treatments and in both habitats (Figs. 4*a–4j*). ΣSAFAs/C, but not ΣMUFAs/C, showed significant differences between the two habitats for both NP and NPSi treatments, whereas ΣPUFAs/C differed significantly between the two habitats for the NPSi treatment. Small but steady increases with time were observed in Σω3/C PUFA but not in Σω6/C PUFA in both habitats (Figs. 4*d*, 4*e*, 4*i*, and 4*j*). Significantly higher levels of Σω6/C PUFA were found in the Norwegian Sea in both the NP and NPSi treatments (Table 1). The diatom-indicating PA/C also showed significantly higher levels in the Norwegian Sea than in the southern Baltic Sea in both treatments, but in the NP treatments, the contents leveled off during the last half of the experiment (Figs. 5*a* and 5*e*). EPA/C and DHA/C were rather stable and only small increases were observed during the last part of the experiment, particularly in the southern Baltic Sea (Figs. 5*b*, 5*c*, 5*f*, and 5*g*). No significant differences in EPA/C and DHA/C between the two habitats were found in any of the treatments. However, during the last 4 days when nutrient additions had been stopped, a tendency towards higher levels of DHA/C was found in the southern Baltic Sea in both treatments. The

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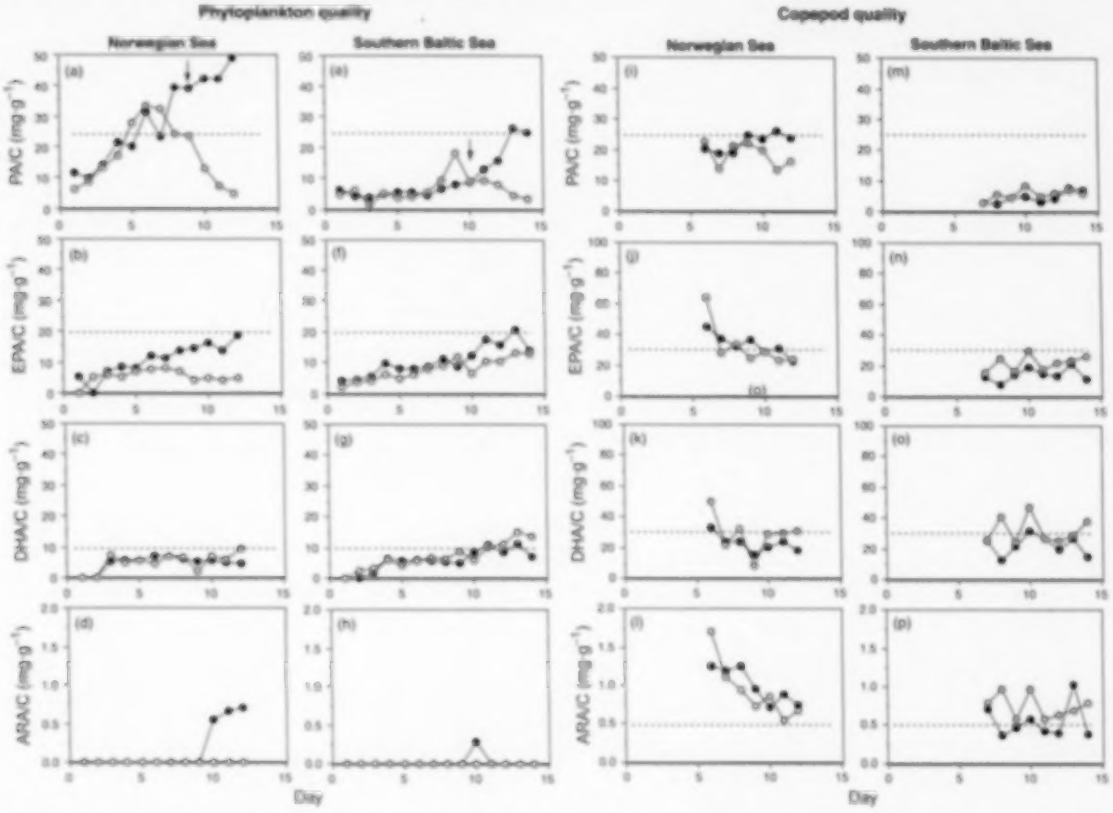
Fig. 5. Content of FA per C: PA/C, EPA/C, DHA/C, and ARA/C versus day in the two mesocosm experiments in (*a–h*) phytoplankton and (*i–p*) copepods. Shaded and solid circles indicate NP and NPSi treatments, respectively. Arrows indicate the end of the nutrient additions. Broken lines make comparison between the two habitats easier.



ARA/C during the last 3 days was doubled in the Norwegian Sea compared with the traces found during day 10 in the southern Baltic Sea (Figs. 5*d* and 5*h*). The ratio ΣPUFAs/ΣSAFAs increased slowly in both habitats, and in the NP treatment, significantly higher levels were found in the southern Baltic Sea (Figs. 6*a* and 6*d*). The Σω3/Σω6 ratios increased with time and significantly higher levels were found in both treatments in the southern Baltic Sea (Figs. 6*b* and 6*e*). The ratios were particularly high during the last part of the experiment in the southern Baltic Sea. When it was possible to calculate the DHA/ARA ratios in the phytoplankton, they were as low as 7 in the Norwegian Sea and as high as 30 in the southern Baltic Sea (Figs. 6*c* and 6*f*).

Concerning the quality of copepod FAs, both ΣSAFAs/C and ΣMUFAs/C showed significantly higher levels in the Norwegian Sea than in the southern Baltic Sea in both treatments (Figs. 4*k*, 4*l*, 4*p*, and 4*q*; Table 1). ΣPUFAs/C, Σω3/C, and Σω6/C showed higher levels in the Norwegian Sea but the difference was not significant. PA/C showed significantly higher levels in both treatments in the Norwegian Sea but EPA/C was higher only in the NPSi treatments (Figs. 5*i*, 5*j*, 5*m*, and 5*n*). The copepod PA/C increased significantly with time in NPSi treatments in both habitats, with a rate of 1.0 ($P = 0.046$) and 0.65 mg·g^{-1}·day^{-1} ($P = 0.015$), respectively. EPA/C in the Norwegian Sea decreased significantly, whereas EPA/C in the southern Baltic Sea and DHA/C in both habitats showed no significantly changes with time. ARA/C also showed higher levels in the Norwegian Sea in the NPSi treatment, whereas DHA/C varied more and was not significantly different between the two habitats (Figs. 5*k*, 5*l*, 5*o*, and 5*p*). ARA/C decreased in the Norwegian Sea but stabilized the last 3 days above 0.5 mg·g^{-1} when ARA appeared in phytoplankton (Figs. 5*d* and 5*l*). The ratios ΣPUFAs/ΣSAFAs in both treatments and DHA/ARA in the NPSi treatment were significantly higher in the southern Baltic Sea, whereas the ω3/ω6 ratios were not significantly different between the two habitats (Figs. 6*g–6l*).

The relationships between phytoplankton and copepods in PA, EPA, and DHA concentrations (μg·L^{-1}) or normalized to C (mg·g^{-1} C) showed different patterns in the Norwegian Sea and southern Baltic Sea mesocosms (Fig. 7). In the Norwegian Sea, the copepod FA concentrations versus phytoplankton FA concentrations were more or less stable, whereas a tendency toward decreases appeared in the southern Baltic Sea ($P = 0.1$ for EPA) (Figs. 7*a–7f*). Qualitatively, the content of PA/C in copepods versus in phytoplankton showed a small increase in both habitats (slope = 0.23 in the Norwegian Sea, slope = 0.21 in the southern Baltic Sea) (Figs. 7*g* and 7*j*). In the Norwegian Sea, EPA/C decreased and DHA/C increased (slope = –2.3 and 3.6, respectively)

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Fig. 6. Ratios of ΣPUFAs/ΣSAFAs (P/S), Σω3/Σω6), and DHA/ARA versus day in the two mesocosm experiments in (*a–f*) phytoplankton and (*g–l*) copepods. Shaded and solid circles indicate NP and NPSi treatments, respectively. Arrows indicate the end of the nutrient additions. Broken lines make comparison between the two habitats easier.

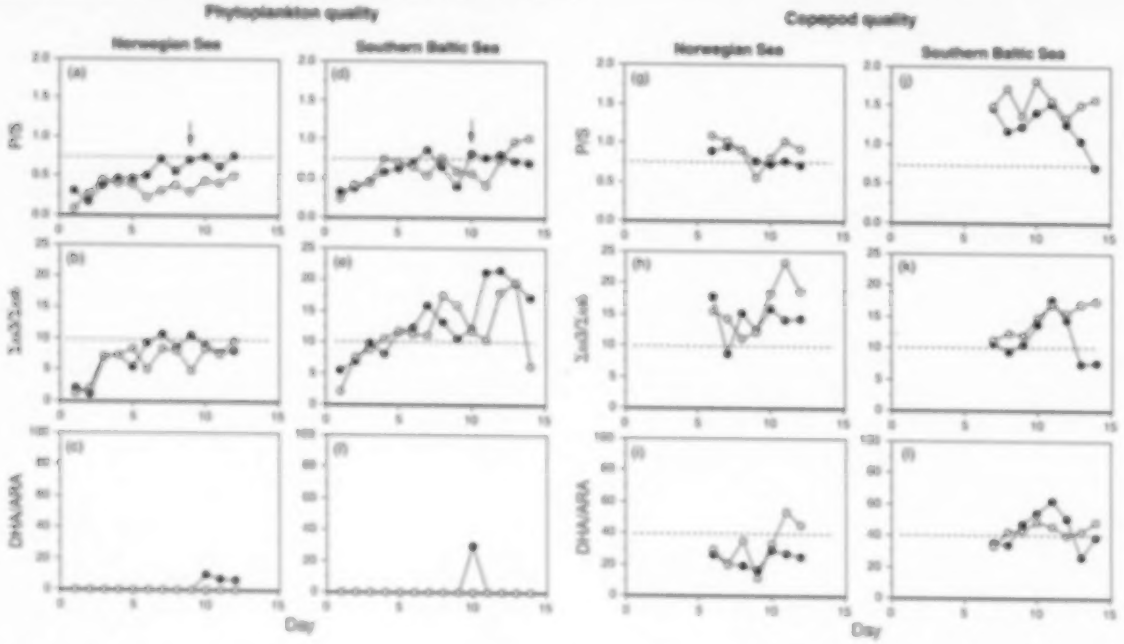
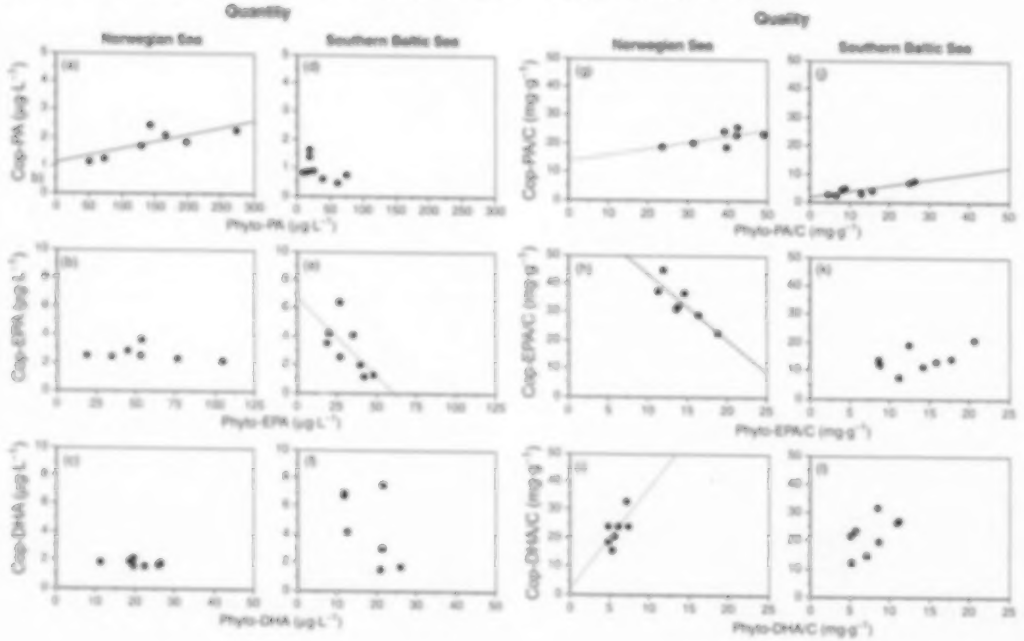


Fig. 7. Results of regression analyses between phytoplankton (Phyto) and copepod (Cop) FA in the mesocosms experiments with NPSi additions. (*a–f*) Concentrations of PA, EPA, and DHA; (*g–l*) content of PA/C, EPA/C, and DHA/C. Whole lines indicate significance at $P = < 0.05$ and broken lines significance at $P \leq 0.1$. Significant regressions: (*a*) $y = 0.005x + 1.09$ ($R^2 = 0.58$, $P = 0.048$); (*e*) $y = -0.110x + 6.71$ ($R^2 = 0.45$, $P = 0.070$); (*g*) $y = 0.228x + 13.51$ ($R^2 = 0.43$, $P = 0.110$); (*h*) $y = -2.339x + 66.96$ ($R^2 = 0.73$, $P = 0.015$); (*i*) $y = 3.571x + 2.05$ ($R^2 = 0.44$, $P = 0.103$); (*j*) $y = 0.209x + 1.84$ ($R^2 = 0.79$, $P = 0.003$).



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(Figs. 7*h* and 7*i*). The relationships between phytoplankton and copepod EPA/C and DHA/C in the southern Baltic Sea mesocosm showed tendencies to increasing slopes (not significant) (Figs. 7*k* and 7*l*).

Discussion

Phytoplankton C (seston) showed a stronger response to the NPSi additions in the Norwegian Sea than to those in the southern Baltic Sea, whereas the Chl response had similar patterns in both habitats. This resulted in opposite developments of the Chl/C ratio throughout the experiments: decreasing in the Norwegian Sea but increasing in the southern Baltic Sea. An often used average value of Chl/C ratio in phytoplankton is 0.02 (*m/m*) or C/Chl = 50 (Vollenweider 1969; Ahlgren 1983). Our results indicate a higher proportion of detritus C in the Norwegian Sea (Chl/C < 20 $mg \cdot g^{-1}$) after the nutrient additions were stopped (day 9), whereas in the southern Baltic Sea, a higher proportion of detritus C occurred at the start of the experiment. The maximum response of the Chl concentration to the nutrient additions appeared 2 days earlier in the Norwegian Sea than in the southern Baltic Sea (day 6 versus day 8) despite the fact that water temperature was lower, 12–13 and 15–19 °C, respectively. A probable reason is that there was a high abundance of diatoms in the Norwegian Sea experiment that quickly responded to the Si additions.

The same pattern as for phytoplankton C was found in the FA concentrations in the two habitats, i.e., a stronger response in the Norwegian Sea NPSi mesocosm. In contrast, the responses in the NP mesocosms were smaller and often leveled off when the nutrient additions were stopped. It is very likely that the different algal flora at the start of the experiment was responsible for this difference. The significantly higher levels of the diatom-indicating PA in the phytoplankton, both quantitatively and qualitatively, confirmed the dominance of diatoms in the Norwegian Sea. EPA is also common in diatoms (Groth-Nard and Robert 1993), and the steep increase of EPA in the NPSi treatments of the Norwegian Sea mesocosm indicates a higher growth of diatoms than in the southern Baltic Sea. The high content of DHA in the southern Baltic Sea phytoplankton during the latter part of the experiment and the absence of ARA, which contribute to the high Σω3/Σω6 ratios, indicate growth of flagellates. This is of vital importance because many flagellates do not produce thiamin, whereas many green algae and diatoms do (Brown et al. 1999; Pinto et al. 2003). Warmer winters during the 1990s may have contributed to similar shifts in phytoplankton composition in the southern Baltic Sea because water column stratification was modified (Wasmund et al. 1998). After a warm winter with no or little ice cover, the spring diatom bloom is usually less pronounced.

The concentrations of ΣSAFAs and ΣMUFAs in copepods were not much different between the two habitats in spite of three times higher copepod abundance in the southern Baltic Sea. The reason for this is that the individual size of the dominant copepod *Acartia* in the southern Baltic Sea was much smaller and they did not increase in number and size. The steep decrease in all FA groups, including EPA, DHA, and ARA, in copepods during the last 4 days of the experiment in the southern Baltic Sea was supported by examination of copepod abundance. The copepods started to die off when the nutrient additions were stopped (Van Nieuwerburgh 2004). In contrast, the higher levels of ΣSAFAs/C and ΣMUFAs/C in copepods in the Norwegian Sea mesocosms show that these copepods had a higher lipid content than did copepods from the southern Baltic Sea. The qualitatively higher levels of PA/C in copepods from the Norwegian Sea indicate that the copepods also utilized the greater access to diatom food in the Norwegian Sea. Ratios such as ΣPUFAs/ΣSAFAs, Σω3/Σω6, and DHA/ARA also indicate qualitative aspects. The abnormally high ΣPUFA/ΣSAFA ratios (>1) in the southern Baltic Sea copepods also indicate that the animals were leaner in this habitat. However, the Σω3/Σω6 ratios of the copepods were not significantly different in the two habitats, whereas the DHA/ARA ratios were significantly higher in the NPSi treatment of the southern Baltic Sea. This indicates an imbalance in the biologically active FAs in the copepods in the NPSi treatment of the southern Baltic Sea mesocosm. Copepods, as probably all animals in general, cannot vary their biochemical composition as much as phytoplankton do, particularly with respect to essential minerals or biochemical substances (Hessen and Andersen 1992). If food is in short supply, a small population can be supported, but if the food quality is low (e.g., an essential substance is missing) even a small population cannot survive and dies off sooner or later. The most probable effect of a deficiency of an essential substance is that this species is replaced by another, more insensitive species that can cope with a lower supply of the essential compound (Olsen 1999). One reason for the decline of copepods in the southern Baltic Sea might be suboptimal levels of ARA, as these were abnormally low in comparison with DHA. According to Fig. 5*p*, the minimum level of ARA in living copepods in the southern Baltic Sea seems to be about 0.5 $mg \cdot g^{-1}$ C, and according to Fig. 6*l*, DHA/ARA ratios >40 seem to be lethal for the same copepod species.

The regressions between some copepod and phytoplankton FAs in the NPSi mesocosms showed different responses between the two habitats (Fig. 7). Quantitatively, the accumulation of PA in the Norwegian Sea copepods was small but significant, whereas the concentrations of EPA and DHA were stable. That suggests that the conservative PA was stored, whereas the copepods used EPA and DHA at about the same rates as they were consumed. In the southern Baltic Sea, the decreasing concentration of EPA and DHA in the copepods showed that the copepods could apparently not utilize the increasing concentration of phytoplankton EPA and DHA. This suggests that some specific FA or other substance was lacking, which resulted in the dramatic death of the copepods during the last 3 days of the experiment.

Considering the quality aspect of the FAs, the slope of copepod PA/C versus phytoplankton PA/C was about the same (0.2) in the two habitats and can be interpreted as a normal intake of the abundant copepod species. It cannot be considered as retention, since the slope is <1 (Castell et al. 1994; Olsen 1999). PA is an inert FA that is not required as any essential compound in animals and does not seem to enter the ordinary desaturation pathways (Sargent and Falk-Petersen 1988; Dalsgaard et al. 2003). Also, no elongation

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seemed to take place, since 18:1ω7 FA was low (10–20 times less than PA) in copepods compared with the phytoplankton food and no ω7 FA with 20 or 22 C were detected in the copepods. In contrast, the higher increase of copepod DHA/C in relation to phytoplankton DHA/C (slope > 1), which partly corresponds to the decrease in EPA/C, indicates both selective retention and chain elongation. The abundant calanoid copepods in our experiment were mainly herbivorous taxa and should therefore be capable of elongating and desaturating EPA into DHA (Brenner 1989). Both EPA/C and DHA/C did not show a significant linear response in the southern Baltic Sea, but EPA/C was lower than DHA/C, in spite of a reversed order in the phytoplankton food. This suggests that the copepods received their DHA mainly from retention and not from conversion from EPA. It seems as if the *Acartia* species in the southern Baltic Sea are mainly omnivorous or that the abundance of DHA was high enough to satisfy the need. Because of the many modifications of essential FAs such as EPA and DHA that can take place in animals, an inert FA such as PA is more appropriate to use as a trophic marker than EPA or DHA.

The importance of ω3/ω6 ratios in the diets for copepods has been stressed earlier (e.g., Jónasdóttir 1994; Jónasdóttir et al. 1995) but not DHA/ARA ratios. However, copepod ARA data are rare in the literature, possibly because its physiological role in copepods is not known. The highest egg production by *Acartia tonsa* was found when the ω3/ω6 ratio in the microalgal food (*Rhodomonas*) was 25 (Jónasdóttir 1994). The optimum ratio for egg production seems to be rather narrow because significantly lower egg production was observed by Jónasdóttir when this ratio was 30. Egg production in the copepods *Acartia hudsonica* and *Temora longicornis* (Long Island Sound, USA) was highest when the estimated ω3/ω6 and DHA/ARA ratios were 6 and 3.2, respectively, in the POC (Jónasdóttir et al. 1995). In addition, copepod ω3/ω6 and DHA/ARA ratios seem to vary with season depending on the available food items. In calanoid copepods, ω3/ω6 ratios in wax esters were reported to vary between 8 and 15 (herbivores) and between 8 and 9 (omnivores) during summer and autumn, and in eggs from *Calanus finmarchicus*, the ω3/ω6 and DHA/ARA ratios were 11 and 15, respectively (Sargent and Falk-Petersen 1988). In a feeding experiment (10 days) with adult *A. tonsa*, the ω3/ω6 ratios in the copepods varied as 5, 7, and 8 when they were fed with *Isochrysis* (haptophyte), *Heterocapsa* (dinoflagellate), and *Thalassiosira* (diatom), respectively, whereas the DHA/ARA ratios varied as much as 18, 170, and 5, respectively (Stottrup et al. 1999). This indicates that some copepods, presumably herbivores, seem to cope with very low ARA contents in their bodies at least for shorter periods. Unfortunately, no information about the reproductive success of these three diet groups was given. In a review on copepod feeding and production, Kleppel (1993) concluded that copepod diets tend to be diverse, thereby optimizing a nutritionally complete ration. It is quite reasonable that high diversity in food items is a general phenomenon in nature and might be a prerequisite for physiologically optimal diets that promote good health in most animals, including humans. Sargent (1995) suggested that optimum levels of the ω3/ω6 ratio should be between 5/1 and 10/1 in broodstock diets and in fish eggs. An optimum level of DHA/ARA ratios for copepods is difficult to establish because these ratios probably vary a great deal among species depending on the capability of converting short-chain ω3 and ω6 FAs into the longer biologically active FAs. In light of the literature cited above, our DHA/ARA ratios >40 in the southern Baltic Sea copepods can be considered as abnormally high.

DHA, with its six double bonds, is particularly vulnerable to oxidative damage compared with the shorter ω3 PUFA (Crawford et al. 1999). The high content of DHA (≥10 mg·g C^{-1}) compared with the lack of ARA in the southern Baltic Sea phytoplankton might provide risks for peroxidative injuries higher up in the food web, particularly when low levels of thiamin in phytoplankton and astaxanthin in copepods also have been documented (Andersson et al. 2003; Van Nieuwerburgh 2004; Wänstrand 2004). When copepods have access to a high diversity of phytoplankton organisms, especially when green algae are present, they produce more astaxanthin than copepods grazing on low-diverse phytoplankton (Van Nieuwerburgh et al. 2004*b*). Particularly green algae contain high amounts of carotenoids that are close to astaxanthin in molecular structure, such as β-carotene, alloxanthin, and zeaxanthin (Jeffrey and Vesk 1997). Cryptophytes contain alloxanthin, but dinoflagellates and diatoms contain only traces of carotenoid precursors of astaxanthin (Jeffrey and Vesk 1997). In addition, the lack of ARA in the southern Baltic Sea phytoplankton probably leads to high ω3/ω6 and DHA/ARA ratios for many herbivorous and omnivorous animals in the Baltic Sea salmon food web. Unfortunately, no recent FA data are available for the Baltic Sea salmon diet, which consist mainly of sprat (*Sprattus sprattus*) and Baltic herring (*Clupea harengus*) (Hansson et al. 2001). However, estimated DHA/ARA ratios from the data in Statens livsmedelsverk (1998) tables show a high value of around 25 in Baltic herring. This might influence the FA pattern in the Baltic Sea salmon, since analyses on phospholipids in M74-defective salmon eggs from Baltic stocks (the River Dalälven) showed high levels of DHA and low levels of ARA, resulting in abnormally high ω3/ω6 and DHA/ARA ratios of 21 and 25, respectively (Pickova et al. 1998). In contrast, ratios of 16 and 19, respectively, were found in healthy Baltic stocks of salmon. However, the ratios from the Baltic non-M74 salmon also seem to be elevated because ω3/ω6 and DHA/ARA ratios in phospholipids in eggs from wild landlocked salmon were only 5 and 4, respectively, and in eggs from reared landlocked stock were 14 and 10, respectively (Pickova et al. 1999). In addition, overproduction of DHA can also result in low production of ARA because of competition with the same desaturase enzymes that are needed in the desaturation process of FAs (Sargent et al. 1995; Ahlgren et al. 1999).

The importance of DHA in vertebrate nutrition has been recognized for many years, whereas the potential importance of ARA has hitherto been largely neglected (Sargent et al. 1995). However, ARA is the major eicosanoid precursor in terrestrial vertebrates, as well in fish, and is therefore essential for most fish, particularly for juveniles (Sargent et al. 1997). ARA also has growth-promoting effects in fish, specific physiological roles in gills and kidneys, and is particularly important in the eicosanoids, which have a major role in egg sheddings, synchronizing ovulation, and spawning.

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thereby avoiding overripening of eggs (Castell et al. 1994; Sargent et al. 1997 and references therein). Whether ARA has any essential functions in copepods is still not known, but recent food web studies give some hints (Kainz et al. 2004). They found that ARA was more accumulated in the size group of mainly copepods than in a group consisting mainly of cladocerans.

In summary, flagellates dominated completely in the southern Baltic Sea at the start of the mesocosm experiment in contrast with the Norwegian Sea, where a higher taxonomic diversity among phytoplankton occurred. At the end of the mesocosms experiment, flagellates still dominated in the NP treatment in the southern Baltic Sea, but small centric diatoms also occurred in the NPSi treatment. In the Norwegian Sea, heavily silicified diatoms dominated in the NPSi mesocosm. This reflects the reported shifts in the phytoplankton flora of the Baltic Sea and supports, together with our results on FAs, the idea that changes in the basic food web may contribute to the development of M74. Diatoms have gradually been replaced by flagellates that contain high amounts of DHA, low levels of carotenoids, and no thiamin and ARA. These flagellates contain high amounts of the ω3 PUFA DHA and no or only traces of the ω6 PUFA ARA and they usually do not produce thiamin. Diatoms contain both ω3 and ω6 FAs and produce thiamin and some carotenoids. In the mesocosm experiments, we found highly elevated ω3/ω6 ratios in phytoplankton from the southern Baltic Sea compared with the Norwegian Sea control, where the M74 syndrome is not found. In copepods from the southern Baltic Sea mesocosms, ARA was very low compared with DHA, resulting in an abnormally high DHA/ARA ratio of about two times higher than in the Norwegian Sea. In the Norwegian Sea, the copepods probably got their DHA content (DHA/C) from both elongation of EPA and retention from the food. In the southern Baltic Sea, the copepods seemed to get their DHA mainly from retention. No retention of PA was shown in the copepods.

We conclude that the high ω3/ω6 ratio of the southern Baltic Sea phytoplankton most probably contributed to the abnormally high DHA/ARA ratio in the copepods. This indicates an imbalance in the basic food web in the southern Baltic Sea and may have implications for the whole food web including the pray fish of salmon. Whether this also induces some salmon females to produce defective eggs and fish larvae remains to be proven. The initial stages of the M74 syndrome in the salmon fry are mostly neurological, and neural tissue contains high amounts of DHA. Therefore, elevated levels of DHA with its six double bonds at the expense of ARA, together with low levels of antioxidants, may increase the risk for production of harmful free radicals and peroxidative injuries. Our mesocosm study shows that the FA composition at the base of the food web is sensitive to changes in the environment. To address the possible involvement of changes in FAs in the incidence of M74, thorough investigations of the FA contents in all Baltic salmon food web levels in the open Baltic Sea are necessary.

Acknowledgements

We thank S. Tengblad for expert help in performing the GLC analyses of the FAs, J. Johansson for analyses of C and N contents, S. Karlsson for statistical advice, and I. Ahlgren for valuable suggestions to improve the manuscript. We also thank two unknown referees whose critiques and suggestions improved this paper. This study was financed by the Knut and Alice Wallenberg Foundation, the Norsk Hydro Research Foundation, and the Ebba and Sven Schwartz' Foundation.

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Estimates of egg deposition and effects of lake trout (*Salvelinus namaycush*) egg predators in three regions of the Great Lakes

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Abstract: Excessive mortality from spawning through the first year of life is likely responsible for recruitment failures in many lake trout (*Salvelinus namaycush*) populations. We evaluated relationships between interstitial predator species and lake trout egg deposition in three regions of the Great Lakes. The introduced crayfish *Orconectes rusticus* dominated the nearshore zone in northern Lake Michigan, whereas other sites in Lake Michigan and Parry Sound were dominated by native *Orconectes propinquus*. In Lake Champlain, sculpin (*Cottus* spp.) were the most common interstitial predator, yet densities were comparable with those in Lake Michigan. Predator densities in Lake Michigan (mean ± standard error = 15.5 ± 0.4 predators·m^{-2}) were twice those in Lake Champlain or Parry Sound (6 ± 1 predators·m^{-2}). Densities of eggs were higher in Lake Champlain (2994.1 ± 398.3 eggs·m^{-2}) and Parry Sound (454.7 ± 36.3) than in Lake Michigan (7.5 ± 1.3; Kruskal–Wallis, $P \leq 0.05$). Ratios of eggs to predator provide simple measures of the effectiveness of lake trout rehabilitation efforts. The average ratio calculated from 10 successful spawning locations is 190 eggs·predator^{-1}·m^{-2}. Ratios may help establish target egg densities for restoration efforts and the concentration of spawning adults required to allow survival to the fry stage.

Résumé : L'insuccès du recrutement chez plusieurs populations de touladis (*Salvelinus namaycush*) s'explique par une mortalité excessive après la fraye et au cours de la première année de vie. Nous avons évalué les relations entre les espèces de prédateurs dans la zone interstitielle et la ponte d'oeufs de touladis dans trois régions des Grands Lacs. L'écrevisse introduite *Orconectes rusticus* domine la zone proche du rivage dans le nord du lac Michigan, alors que l'espèce indigène *Orconectes propinquus* prédomine dans les autres sites du lac Michigan et à Parry Sound. Au lac Champlain, les chabots (*Cottus* spp.) sont les prédateurs les plus communs dans la zone interstitielle, mais les densités sont comparables à celles du lac Michigan. Les densités de prédateurs au lac Michigan (moyenne ± écart type = 15,5 ± 0,4 prédateurs·m^{-2}) sont deux fois plus élevées que celles au lac Champlain ou à Parry Sound (6 ± 1 prédateurs·m^{-2}). Les densités d'oeufs sont plus élevées au lac Champlain (2994,1 ± 398,3 oeufs·m^{-2}) et à Parry Sound (454,7 ± 36,3) qu'au lac Michigan (7,5 ± 1,3; test de Kruskal–Wallis, $P \leq 0,05$). Le nombre d'oeufs par prédateur fournit une mesure simple de l'efficacité des efforts de réhabilitation du touladi. Le rapport moyen calculé dans 10 sites de fraye « réussie » est de 190 oeufs·prédateur^{-1}·m^{-2}. Ces rapports peuvent servir à fixer les densités d'oeufs à atteindre dans les efforts de restauration et à déterminer les concentrations d'adultes reproducteurs nécessaires pour permettre la survie jusqu'au stage alevin.

[Traduit par la Rédaction]

Introduction

Current research suggests that excessive mortality between the time of spawning in the fall and the first year of life is likely responsible for recruitment failures in lake trout (*Salvelinus namaycush*) populations throughout most of the Great Lakes (Jones et al. 1995; Savino et al. 1999). By the 1960s lake trout in all of the lakes except Lake Superior and the Parry Sound portion of Lake Huron were extirpated due to overfishing and sea lamprey (*Petromyzon marinus*) predation. With exceptions for Lake Superior and Parry Sound, stocking during the last four decades has generally failed to produce substantial recruitment. In Lake Michigan, we know that lake trout are not recruiting to the fishery, yet have

Received 17 June 2004. Accepted 11 April 2005. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on 17 September 2005.
J18186

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 doi: 10.1139/F05-141 © 2005 NRC Canada

some evidence of reproductive efforts (eggs, fry, and rarely young-of-the-year). The extent of these efforts in relation to other more successful systems has not been evaluated.

Relative abundance and age composition estimates are often the only parameters evaluated when determining the status of lake trout populations. A comprehensive synthesis of gillnet catch rates and their implied relation to adult abundance established the metric (50 lake trout·305 m^{-1} of gillnet; range, 17–135) that many managers currently target to establish spawner abundance levels capable of producing measurable recruitment and self-sustaining stocks (Selgeby et al. 1995). As a result, investigations into the spawning potential of lake trout have primarily focused on measurements of the abundance of spawning adults (Holey et al. 1995; Selgeby et al. 1995) on specific reefs with only occasional estimates of egg deposition (Peck 1986; Perkins and Krueger 1995; Chotkowski and Marsden 1997, 1999). Evaluations of the influence of mortality factors in the early life stages of lake trout life history have been nearly non-existent, and the relationships to spawning stock size and habitat use have not been established (Horns and Magnuson 1981; Kelso 1995).

To truly characterize mechanisms behind recruitment failures and evaluate reproductive potential, estimates of adult abundance during spawning are not sufficient. For measurable natural reproduction to occur, enough eggs must be deposited to survive interstitial predation and develop over the winter into emergent fry, after which they must escape the gauntlet of piscivorous predators offshore (Gunn 1995). The inability to reach any of these stages results in recruitment failure. In Lake Ontario, Fitzsimons et al. (2002) measured predator abundance levels that were high enough to cause mortality of all eggs when deposition rates were low. When egg deposition increased, predator impacts were reduced. Our current knowledge of predation losses in the early life history of lake trout is based almost entirely on laboratory studies and modeling efforts (Jones et al. 1995; Savino et al. 1999); these indicate severe consequences to recruitment but have yet to be validated in the field.

The potential effects of native and non-native interstitial predators on lake trout recruitment are strongly dependent on the density of naturally deposited eggs, the species composition, density and size of predators, and their underlying relationships (Jones et al. 1995; Savino et al. 1999). Each species of predator has a finite ability to consume eggs that is likely regulated by their size (Evans et al. 1987; Post and Lee 1996) and water temperature (Thornton and Lessem 1978; Carline 1987; Hill and Magnuson 1990). The relative importance of egg predation may be even greater now, with the recent invasion of rusty crayfish (*Orconectes rusticus*) and round gobies (*Neogobius melanostomus*), two species known to consume lake trout eggs at higher rates than native crayfish and sculpins (Capelli 1982; Chotkowski and Marsden 1999; Clapp et al. 2001). Moreover, both round gobies and rusty crayfish are documented competitors for food resources and habitat, and are highly fecund when compared with native species (Capelli 1982; Olsen et al. 1991; Chotkowski and Marsden 1999). Rusty crayfish are known to interbreed with *Orconectes propinquus* (Capelli and Capelli 1980). Site-specific estimates of predator species abundance, species composition, and size distributions would better define the effects of predation on lake trout recruitment success.

The goals of our study were as follows: (*i*) determine the number of naturally deposited lake trout eggs surviving to overwinter on several spawning reefs in Lake Michigan, Parry Sound (Lake Huron), and Lake Champlain; (*ii*) determine the density, species composition, and size ranges of interstitial predators on these reefs; and (*iii*) evaluate egg and predator densities to determine target egg-to-predator ratios that will lead to emergence of lake trout fry, a precursor to measurable recruitment.

Methods

We measured lake trout egg densities after exposure to interstitial predation and prior to the overwinter period in three diverse large lake systems (Lake Michigan, Parry Sound (Lake Huron), and Lake Champlain; Fig. 1). Systems were selected because they provide extensive habitat for lake trout reproduction, and are focal areas for lake trout restoration efforts (Holey et al. 1995; Reid et al. 2001; Ellrott and Marsden 2004). Within a given system, spawning sites were identified through reconnaissance diving surveys of habitat, past estimates of lake trout egg deposition, and existing knowledge of sites known to attract spawning lake trout in the fall. Once selected, divers surveyed entire reefs to find areas with substrate, slope, and depth characteristics appropriate for attracting spawning lake trout (Fitzsimons 1994; Marsden et al. 1995).

In Lake Michigan, no remnant stocks of lake trout remain, target stocking levels have never been achieved, lamprey populations (while significantly lower than during the pre-control period) continue to kill significant numbers of lake trout, and in some areas, commercial and recreational exploitation rates have remained high (Holey et al. 1995; Bronte et al. 2003*b*). When measured, egg deposition has been low or absent (Marsden 1994; Holey et al. 1995; Marsden and Janssen 1997), and the relationships between egg density and interstitial predators have not been evaluated. Most of the potential lake trout spawning habitat is found in the northern part of the lake, which is the focal area of our investigations (Dawson et al. 1997). Historically, lake trout co-existed with an interstitial community that included native crayfish (*Orconectes propinquus* and *Orconectes virilis*) and sculpin (*Cottus bairdi* and *Cottus cognatus*). However, the nearshore reef communities in Lake Michigan have recently been dramatically altered by the invasion of exotic egg predators (i.e., rusty crayfish and round gobies). For analyses, we split northern Lake Michigan into four regions: a protected bay with large populations of native predators (Ingalls Point in Grand Traverse Bay), a nearshore region with small numbers of native predators (Leeland), three nearshore locations with large populations of exotic predators, and five offshore locations with native predator communities (Fig. 1). Much of the potential lake trout spawning habitat in northern Lake Michigan is represented by the onshore exotic predator and offshore native predator habitats. The offshore sites were Dahlia Shoal, Middle Ground, Hog Island Reef, Gull Island Shoal, and Richards Reef. Of these locations, only Gull Island Shoal and Richards Reef had been stocked in the past 15 years. The nearshore locations were Bay Harbor (Little Traverse Bay – South), Fisherman's Island, and a man-made crib structure (Little Traverse Bay –

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Fig. 1. Map of the northeastern United States and southern sections of the Canadian provinces Ontario and Québec indicating regions evaluated and study-site locations within regions.

North). Of the nearshore locations, Bay Harbor (Little Traverse Bay – South), Fisherman's Island, and Leeland have been stocked in the past 15 years.

In contrast with Lake Michigan, lake trout populations in Parry Sound (Lake Huron) are now self-sustaining, and managers have declared success in rehabilitation efforts (Reid et al. 2001). Stocking was discontinued in 1998, remnant stocks of naturally reproducing lake trout remain, and older (>8 years) spawning lake trout are abundant in the population (Reid et al. 2001). This region serves as an important reference point for evaluating successful levels of egg deposition in the presence of native egg predators. In Parry Sound, adult fish are likely depositing sufficient numbers of eggs on spawning reefs to overcome predation, yet the dynamics of egg deposition and egg predation are not well documented. Five nearshore sites (Davy, Bar, Mowatt, Horse, and Mary islands), identified as active lake trout spawning reefs in previous investigations, were selected for this project (J.D. Fitzsimons, personal communication; Fig. 1).

Lake Champlain represented an intermediate case, in which predators are mainly native species, there are no remnant stocks of lake trout, and only 5%–10% of adult lake trout are naturally produced (Ellrott and Marsden 2004). Key factors implicated in lake trout recruitment failures throughout the Great Lakes (e.g., alewives, Krueger et al. 1995; Fitzsimons et al. 1999; high contaminant burdens, Fitzsimons 1995*b*; and excessive overexploitation, Stanley et al. 1987; Holey et al. 1995) are absent in Lake Champlain, creating a unique opportunity to evaluate the relative importance of egg predation in limiting recruitment. We selected three nearshore sites based on reconnaissance diving surveys and anecdotal stories from local fisheries managers and anglers: Grand Isle, Arnold Bay, and Whallon Bay. An additional three sites were identified based on habitat characteristics alone (Fitzsimons 1994; Marsden et al. 1995): Saxton Cove, Iron Bay, and Coast Guard Station (Fig. 1).

We used egg bags to collect lake trout eggs and their predators in the fall (Perkins and Krueger 1994, 1995; Fitzsimons 1995*a*). Each egg bag was constructed from a 0.5 m deep nylon mesh material (3-mm mesh) attached to a polyvinyl chloride ring (0.35 m in diameter or 0.96 m^2 in area). Divers deployed nets by excavating a 40 to 50 cm deep hole, placing the net in the hole, and back filling with the removed substrate. Egg bags ($N = 60$) were buried at each location prior to the spawning season (August–September). The bags were placed in pairs along either side of a 30 m long marked line at 1-m intervals. The bags were positioned at the top of the drop-off at each location. Bags were retrieved after the spawning season (late October – early November). Egg densities reported in this investigation represent the number of eggs surviving predation approximately 4 weeks past peak spawning (late November – early December). Divers would carefully remove the substrate from inside the bag, while preventing interstitial predators from escaping. Predator escapes were rare, readily observed, and recorded by the divers. Recovered bags were brought to the lab where all eggs and predators were counted. We calculated the average number of eggs per square metre and the associated

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variance (standard error (SE)) for each site and lake. Interstitial predators were identified, counted, weighed (g), and measured (mm). Total lengths were recorded for all fish species, and carapace lengths were recorded for all crayfish species. For each site, we estimated the density per square metre of individual predator species and of all predators combined. We only included individuals that were large enough to prey on lake trout eggs. Crayfish with carapace lengths greater than 19 mm and sculpin and round gobies greater than 42 mm total length were considered viable lake trout egg predators (Chotkowski and Marsden 1999; Fitzsimons et al. 2002).

Egg abundance, predator abundance, and predator length data did not meet the assumptions of normality or homogeneity of variance. Log and square root transformations did not normalize the data. Therefore we use the non-parametric Kruskal–Wallis (K–W) one-way analysis of variance by ranks to evaluate differences in egg and predator density among sites and regions. After determining significance in the overall test, multiple comparisons within groups were completed by calculating the critical difference value using z at $\alpha = 0.05$ for $k(k-1)$ comparisons (Siegel and Castellan 1988). We also calculated the average length of each predator species by site and lake, and estimated the variance (SE).

To establish a measure of the interaction between egg deposition and predator abundance and to determine the relationship to fry emergence in the spring, we estimated the number of lake trout eggs deposited per predator within a square metre area at each location. Estimates of eggs per predator were determined for sites with and without fry emergence in the spring.

Emergence in the spring was measured using 10–30 emergent fry traps at each site (J.E. Marsden, B.J. Elfrott, R.M. Claramunt, J.L. Jonas, and J.D. Fitzsimons, unpublished data). Traps were checked weekly and deployed for 6–10 weeks beginning in mid-April, when ice no longer prohibited access to sites. The emergence of fry provided a measure of success in surviving predation and the overwinter period.

Results

Predator species composition, density, and size

Systems that were successful in producing measurable fry emergence tended to have lower densities of interstitial predators. Lake Champlain and Parry Sound both had low predator densities, averaging 6 predators·m^{-2} (range, 2–11 m^{-2}; K–W, $P > 0.05$), which was less than one-half that of Lake Michigan at 15.5 ± 0.4 predators·m^{-2} (range, 8–27 m^{-2}; K–W, $P \leq 0.05$; Table 1).

We observed eight native and two exotic species in egg collection bags from sites in Lake Michigan. Potential egg predators included four fish species (mottled and slimy sculpin, common white suckers (*Catostomus commersoni*), and longnose suckers (*Catostomus catostomus*)) and two crayfish (*O. propinquus* and *O. virilis*); we also collected johnny darter (*Etheostoma nigrum*), longnose dace (*Rhinichthys cataractae*), smallmouth bass (*Micropterus dolomieui*), and mudpuppies (*Necturus maculosus*). Darters, dace, and minnows are not considered viable predators: they are gape limited and do not consume lake trout eggs that are relatively large (5–5.6 mm diameter; Auer 1982). Young-of-the-year smallmouth bass were evaluated in the lab and did not consume lake trout eggs (J.D. Fitzsimons, G. Fodor, and B. Williston, unpublished data). The two exotic species, both egg predators, were rusty crayfish and round goby. A large number of longnose dace were collected at one nearshore location (Leeland); though not egg predators, they were placed in the "other" category to illustrate the unique attributes of this location (Fig. 2).

Egg predators accounted for 97% of the interstitial species collected in Lake Michigan and the abundance of predators did not differ between offshore (14.4 ± 0.6 m^{-2}) and nearshore (16.0 ± 0.6 m^{-2}) locations (K–W, $P = 0.08$). Crayfish and sculpin were most abundant, crayfish (9.1 ± 0.3 m^{-2}) being significantly more abundant than sculpin (6.1 ± 0.3 m^{-2}; K–W, $P < 0.001$; Fig. 2). Rusty crayfish and the round goby were found only at nearshore locations, and rusty crayfish (7.9 ± 0.5 m^{-2}) were more abundant than round gobies (0.8 ± 0.2 m^{-2}; K–W, $P < 0.001$; Fig. 2). Average densities of the three species of crayfish differed (K–W, $P < 0.05$); *O. rusticus* was most abundant (7.9 ± 0.5 m^{-2}), followed by *O. propinquus* (4.2 ± 0.2 m^{-2}) and *O. virilis* (2.1 ± 0.2 m^{-2}; Fig. 3). The average density of round gobies was lower (0.8 ± 0.2 m^{-2}) than that of sculpin (5.8 ± 0.3 m^{-2}; K–W, $P < 0.001$). However, sculpin densities were lower at sites with round gobies (2.5 ± 0.2 m^{-2}) when compared with locations where they were absent (7.7 ± 0.4 m^{-2}; K–W, $P < 0.001$). Similarly, *O. propinquus* densities were much lower (0.8 ± 0.2 m^{-2}) at nearshore sites, where they co-existed with rusty crayfish, when compared with sites where they were absent (7.1 ± 1.6 m^{-2}; K–W, $P < 0.001$; Fig. 3).

In Parry Sound we observed eight native species in egg collection bags: five fish (two predators, rock bass (*Ambloplites rupestris*) and sculpin (slimy and mottled), and three non-predators, bluntnose minnow (*Pimephales notatus*), largemouth bass (*Micropterus salmoides*), and smallmouth bass)) and three crayfish (*O. propinquus*, *O. virilis*, and *Cambarus robustus*); no exotic species were observed. Crayfish were most abundant in Parry Sound, accounting for over 71% of the interstitial species collected. *Orconectes propinquus* was more abundant (3.9 ± 0.4 m^{-2}; K–W, $P < 0.05$) than *Cambarus robustus* (0.7 ± 0.2 m^{-2}) and *O. virilis* (0.2 ± 0.1 m^{-2}; K-W, $P > 0.05$; Fig. 3). With the exception of the nearshore zone in northern Lake Michigan, which was dominated by *O. rusticus*, sites in Parry Sound and Lake Michigan were primarily occupied by *O. propinquus* (Fig. 3). Fish accounted for less than 30% of the species collected, and only 50% of these were potential egg predators (Fig. 2). In total, potential egg predators (both fish and crayfish) accounted for 86% of the species collected in egg bags in Parry Sound. Predator abundance levels did not differ among sites in Parry Sound (average = 5.5 ± 0.5 predators·m^{-2}; K–W, $P = 0.057$; Table 1).

Within Lake Champlain, we observed seven native interstitial species in egg bags, including six fish (two predators, rock bass and sculpin (slimy and mottled), and four non-predators, johnny darter, banded killifish (*Fundulus diaphanous*), smallmouth bass, and log perch (*Percina caprodes*)) and one crayfish (*Orconectes limosus*). Egg predators accounted for 91% of the interstitial species collected in egg bags. Unlike Lake Michigan and Parry Sound, sculpin (4.9 ±

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Table 1. Number of egg nets evaluated (*N*), average number of lake trout (*Salvelinus namaycush*) eggs deposited, abundance of all predators large enough to consume lake trout eggs, and the ratio of eggs to predator per square metre in Parry Sound and Lakes Michigan and Champlain.

Site	*N*	Eggs·m^{-2} ± SE	Total predators·m^{-2} ± SE	Egg-to-predator ratio	Fry present
Parry Sound	379	454.7±36.3	5.5±0.5	82.1	y
Bar Island	89	808.2±90.9 x	3.6±0.8 a	222.0	y
Davy Island	89	723.7±100.3 x	4.9±0.8 a	147.5	y
Horse Island	88	214.0±25.1 y	5.6±1.0 a	38.2	y
Mary Island	24	29.9±9.1 z	7.0±2.2	4.3	n
Mowatt Island	89	184.5±28.7 y	7.6±1.1 a	24.3	y
Lake Champlain	294	2994.1±398.3	6.5±0.6	459.5	y
Arnold Bay	15	891.1±283.9 xyz	11.3±3.4 ab	79.1	y
Coast Guard	10	4.2±4.2 x	5.6±2.3 ab	0.8	n
Grand Isle	119	6584.5±875.7 xyz	10.6±1.0 b	618.3	y
Iron Bay	15	1.9±1.3 x	2.8±1.5 ab	0.7	n
Saxton Cove	15	108.9±58.3 xy	1.9±1.3 ab	58.0	y
Whallon Bay	120	680.4±142.7 xyz	2.5±0.5 a	276.0	y
Lake Michigan	1380	7.5±1.3	15.5±0.4	0.5	n
Nearshore	711	20.2±3.6	16.0±0.6	1.3	n
Bay Harbor	198	1.6±0.5 x	12.5±1.0 ac	—	n
Fishermans Island	182	2.9±0.8 x	7.8±0.8 bd	0.1	n
LTB-Crib	123	75.9±13.6	21.0±1.4 acd	3.6	n
GTB-Ingalls Point	160	0.4±0.2 x	25.6±1.5 a	0.01	n
Leeland	48	0.0±0.0 x	15.6±2.3 bcd	—	n
Offshore	669	0.2±0.1	14.4±0.6	0.01	n
Hog Island	169	0.0±0.0 x	10.5±1.0 bcd	—	n
Gull Island Shoal	160	0.8±0.3 x	27.4±1.4 a	0.03	n
Dahlia Shoal	164	0.0±0.0 x	8.8±0.9 bd	—	n
Middle Ground	117	0.0±0.0 x	12.5±1.2 bcd	—	n
Richards Reef	59	0.0±0.0 x	9.5±1.3 bcd	—	n

Note: In the last column, "y" indicates that fry were collected and "n" indicates no fry collected. Significant differences in the number of eggs per square metre and predators per square metre among sites within each of the lakes are indicated by different letters within a category and lake ($\alpha = 0.05$; Kruskal–Wallis multiple comparisons). SE, standard error; —, no data; LTB, Little Traverse Bay crib site; GTB, Grand Traverse Bay.

0.4 m^{-2}) were more abundant than crayfish in Lake Champlain (1.0 ± 0.2 m^{-2}, K–W, $P < 0.001$), accounting for 68% of the species collected in egg bags. Similar to observations from Parry Sound, predator abundance levels in Lake Champlain were generally low, ranging from 2 to 11 m^{-2} (average = 6.5 ± 0.6 predators·m^{-2}). Sculpin were the most abundant predator in Lake Champlain (4.9 ± 0.4 m^{-2}), yet densities were low and similar to those observed in Lake Michigan (5.5 ± 0.2 m^{-2}; K–W, $P > 0.05$). Densities of sculpin in Parry Sound were the lowest (0.7 ± 0.2 m^{-2}; K–W, $P < 0.05$; Fig. 2).

The length (size) of a predator can influence consumption rates. We examined differences in length between species and systems. *Orconectes propinquus* (20.7 ± 0.7 mm) from Parry Sound were larger on average than in Lake Michigan (15.0 ± 0.2 mm; K–W, $P < 0.001$). In Lake Michigan, *O. propinquus* (15.0 ± 0.2 mm) were smaller than *O. rusticus* (19.6 ± 0.3 mm; K–W, $P > 0.05$), and *O. rusticus* from Lake Michigan were similar in size to *O. propinquus* in Parry Sound (20.7 ± 0.7 mm; K–W, $P > 0.05$; Fig. 4). *Orconectes propinquus* were larger (22.7 ± 1.4 mm) when *O. rusticus* was present at a site than when it was absent (14.7 ± 0.2 mm; K–W, $P < 0.001$). *Cambarus robustus* (27.0 ± 1.7 mm), *O. limosus* (24.3 ± 1.5 mm), and *O. virilis* (25.5 ± 0.6 mm) were the largest of the five species of crayfish observed (K–W, $P > 0.05$). However, the density and frequency of occurrence (<2 m^{-2}) of these species was substantially lower than that observed for either *O. rusticus* or *O. propinquus* (>2 m^{-2}; Figs. 3 and 5). In northern Lake Michigan, sculpin were longer (59.4 ± 0.5 mm) than round gobies (54.0 ± 2.6 mm; K–W, $P < 0.001$); this was likely because round goby populations were in the initial stages of invasion during this study and most individuals were young (J.L. Jonas, C.M. Diana, and R.M. Claramunt, unpublished data). The average size of sculpin was larger in Lake Michigan (62.1 ± 0.6 mm) than in Parry Sound (48.6 ± 4.2 mm) or Lake Champlain (49.7 ± 0.7 mm; K–W, $P < 0.05$).

Egg deposition

As expected, densities of eggs surviving to overwinter varied both within and among systems. Average egg densities in Lake Champlain (mean ± SE = 2994.1 ± 398.3 eggs·m^{-2}) and Parry Sound (454.7 ± 36.3; K–W, $P > 0.05$) were significantly higher than those observed in Lake Michigan (7.5 ± 1.3; K–W, $P \leq 0.05$). Low levels of egg deposition occurred at only 5 of the 10 sites we evaluated in northern Lake Michigan, and extremely small numbers were detected at four of the sites (<3 eggs·m^{-2}; Table 1). A man-

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Fig. 2. Proportion of species captured in egg bags in each of the three lakes, including four regions identified in Lake Michigan (Leeland, offshore, Grand Traverse Bay (GTB), and onshore). Native crayfish are considered egg predators and include *Orconectes propinqus*, *Orconectes virilis*, *Orconectes limosus*, and *Cambarus robustus*. Sculpin include mottled sculpin (*Cottus bairdi*) and slimy sculpin (*Cottus cognatus*). The "other" category primarily represents species that are not considered egg predators, including smallmouth bass (*Micropterus dolomieui*), largemouth bass (*Micropterus salmoides*), longnose dace (*Rhinichthys cataractae*), banded killifish (*Fundulus diaphanous*), johnny darters (*Etheostoma nigrum*), log perch (*Percina caprodes*), and bluntnose minnows (*Pimephales notatus*). Predators such as rock bass (*Ambloplites rupestris*), white sucker (*Catostomus commersoni*), longnose sucker (*Catostomus catostomus*), and mudpuppy (*Necturus maculosus*) were also included in the "other" category because they were infrequently observed.

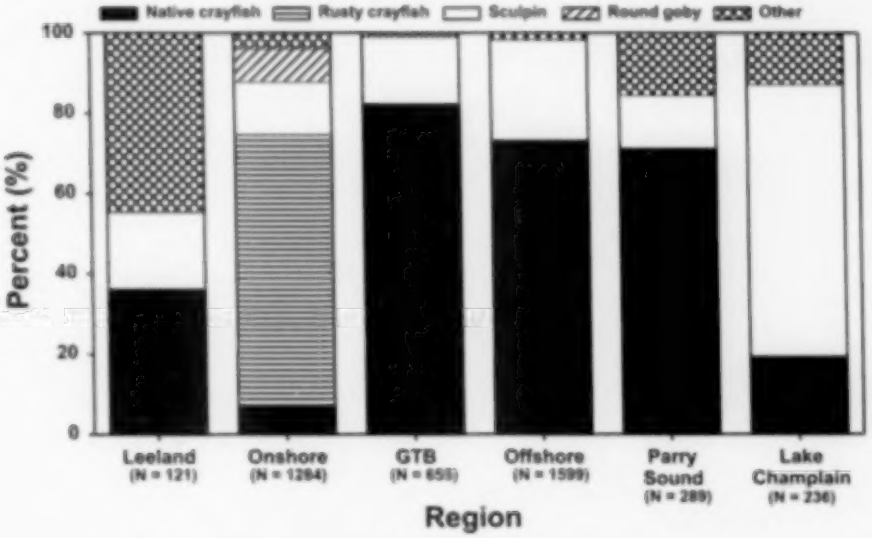
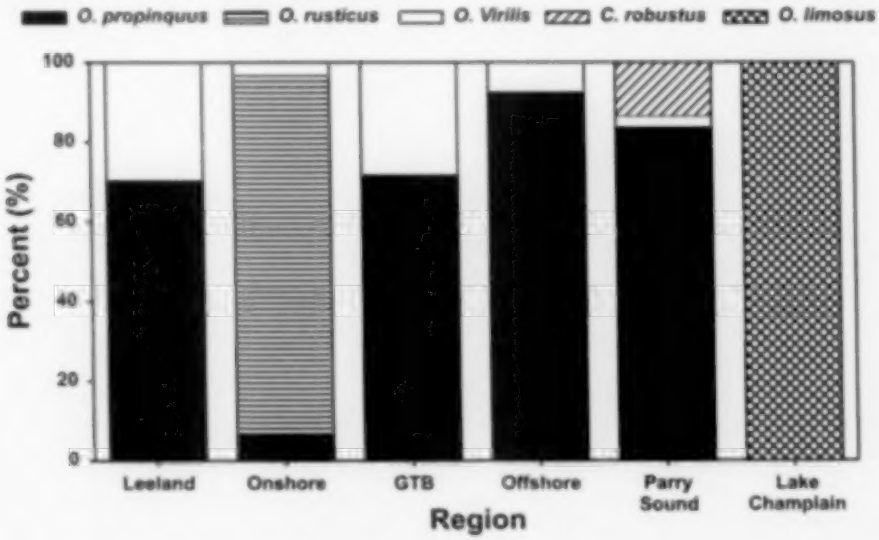


Fig. 3. The percent composition of crayfish species in Lake Champlain, Parry Sound, and four regions in northern Lake Michigan (Leeland, offshore, Grand Traverse Bay (GTB), and nearshore).



made crib located in northern Little Traverse Bay had the highest density of eggs (75.9 ± 13.6 eggs·m^{-2}) when compared with other locations in Lake Michigan (K–W, $P < 0.001$). Lake trout spawned at all five sites in Parry Sound and egg density estimates were generally higher (454.7 ± 36.3 eggs·m^{-2}) than those observed in Lake Michigan (7.5 ± 1.3 eggs·m^{-2}) and lower and less variable than in Lake Champlain (2994.1 ± 389.3; Table 1). Egg densities were highest at Bar and Davy islands (averaging 700–810 eggs·m^{-2}; K–W, $P \leq 0.05$), intermediate at Horse and Mowatt (160–

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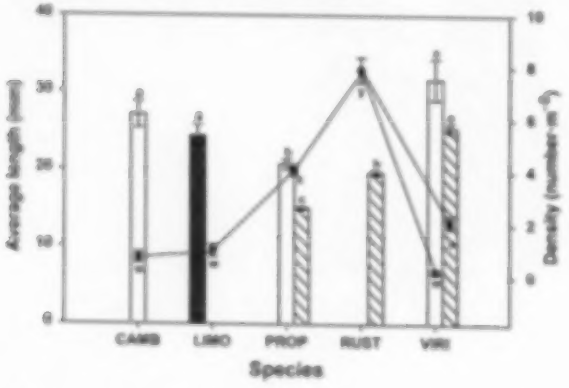
Fig. 4. *Average density* (number·m^{-2}, lines) and average length (mm, bars) summarized by region using sites where crayfish of a given species had established populations. Crayfish species are represented by four letter codes: CAMB, *Cambarus robustus* (Parry Sound sites only); LIMO, *Orconectes limosus* (Lake Champlain sites only); PROP, *Orconectes propinquus* (all Parry Sound and Lake Michigan sites); RUST, *Orconectes rusticus* (onshore Lake Michigan locations only); and VIRI, *Orconectes virilis* (Lake Michigan and Parry Sound). Bars with the same letters are not significantly different within a variable. With the exception of *O. virilis*, the density of predators did not differ between regions within a species group (K–W, $P > 0.05$); therefore, average values are provided for all regions combined. Solid bars represent Lake Champlain, open bars represent Parry Sound, and hatched bars represent Lake Michigan.



215 eggs·m^{-2}; K–W, $P \leq 0.05$), and lowest at Mary (30 ± 9 eggs·m^{-2}; K–W, $P \leq 0.05$; Table 1). We measured the highest density of eggs in Lake Champlain at Grand Isle (6584.5 ± 875.7 eggs·m^{-2}; K–W, $P \leq 0.05$); this location is an extreme outlier when compared with other locations. Egg deposition at other locations in Lake Champlain ranged from 2 to 891 eggs·m^{-2} and was high overall in the lake, with a mean (±SE) of 552.7 ± 102.9 eggs·m^{-2}.

Egg-to-predator ratios

To establish a simple measure of the relationship between egg deposition and predator abundance as they relate to fry emergence in the spring, we estimated the number of lake trout eggs per predator in a square-metre area at each site. Estimates of eggs per predator were compared among sites with and without measured fry emergence (an indication of success in surviving over the winter). Lake Michigan had the lowest ratios (<4 eggs·predator^{-1}·m^{-2}). The average number of eggs per predator in Parry Sound was nearly 20 times that of Lake Michigan and one sixth that of Lake Champlain at 82.1 eggs·predator^{-1}·m^{-2}. Lake Champlain had the greatest average egg-to-predator ratio (459.5 eggs·m^{-2}), nearly 114 times that of Lake Michigan. If the high egg densities at Grand Isle (an apparent outlier) are removed from the analysis, the average ratio becomes similar to Parry Sound at 85 eggs·predator^{-1}·m^{-2}, still nearly 21 times that of Lake Michigan. Fry emergence was not detected at any of the Lake Michigan sites; however, fry emergence was detected at four of five sites evaluated in Parry Sound, and four of six sites evaluated in Lake Champlain, an indication of success in surviving the overwinter period (Table 1; Fig. 5). The lowest number of eggs measured per predator at a successful site was 24 eggs·predator^{-1}·m^{-2} at Mowatt Island in Parry Sound. Successful lake trout populations resulting in fry emergence are likely produced when egg densities are high enough to survive initial predation pressure. In our evaluation, this was generally observed when there were more than 80 eggs·predator^{-1}·m^{-2}. Average ratios across successful sites reveal that egg densities exceeding 190 eggs·predator^{-1}·m^{-2} one month post-spawn will likely succeed in producing emergent fry.

Discussion

Management strategies for lake trout have primarily focused on estimates of adult abundance. We observed significant differences in the number, type, and size of predators, as well as in the number of eggs surviving to overwinter among the systems and sites that we evaluated. Given these differences, we suggest a management strategy that is related to the current strategy, but that shifts the focus to measures of surviving eggs in place of the relative abundance of adults. Interstitial predator communities in northern Lake Michigan were the most lethal. Larger, more abundant exotic species were present at most nearshore locations, while offshore and Grand Traverse Bay communities were similar to those in Parry Sound and were dominated by native crayfish (mainly *O. propinquus*). Total predator densities in Lake Michigan were nearly twice those observed in Lake Champlain or Parry Sound. Sculpin were not only larger in Lake Michigan, but were more abundant when compared with populations in Parry Sound and equal to those observed in Lake Champlain. The second highest potential for predation occurred in Lake Champlain, where overall predator densities were similar to those in Parry Sound, but sculpin were the predominant predator.

Egg deposition was high in Parry Sound and Lake Champlain but barely detectable in Lake Michigan. Egg abundance levels in northern Lake Michigan are among the lowest in the Great Lakes assessed using similar types of gear (Marsden 1994; Fitzsimons 1995*a*; Fitzsimons and Williston 2000), and likely have not increased substantially since early lake trout rehabilitation efforts in the 1970s and early 1980s. Low egg-deposition rates observed in northern Lake Michigan are most likely attributable to a combination of low spawner abundance levels (Bronte et al. 2003*a*) and high interstitial predation rates within the first month after the peak spawning period.

The metric used by most managers to build successful adult stocks (50 spawning lake trout·305 m^{-1} of gillnet; Selgeby et al. 1995), may not represent a suitable target stock size for Lake Michigan, given high levels of interstitial predation. Additionally, stocked lake trout are likely less efficient spawners than wild fish because selective pressures favor hatchery rearing over production in the lake. When data for the current metric were collected (1973–1992), restoration efforts had only been accomplished in Lake Superior, where self-sustaining lake trout stocks had been reestablished. Despite reports of adequate adult abundances at many sites in Lake Michigan, there have been no reports of measurable recruitment. We can infer from the lack of mea-

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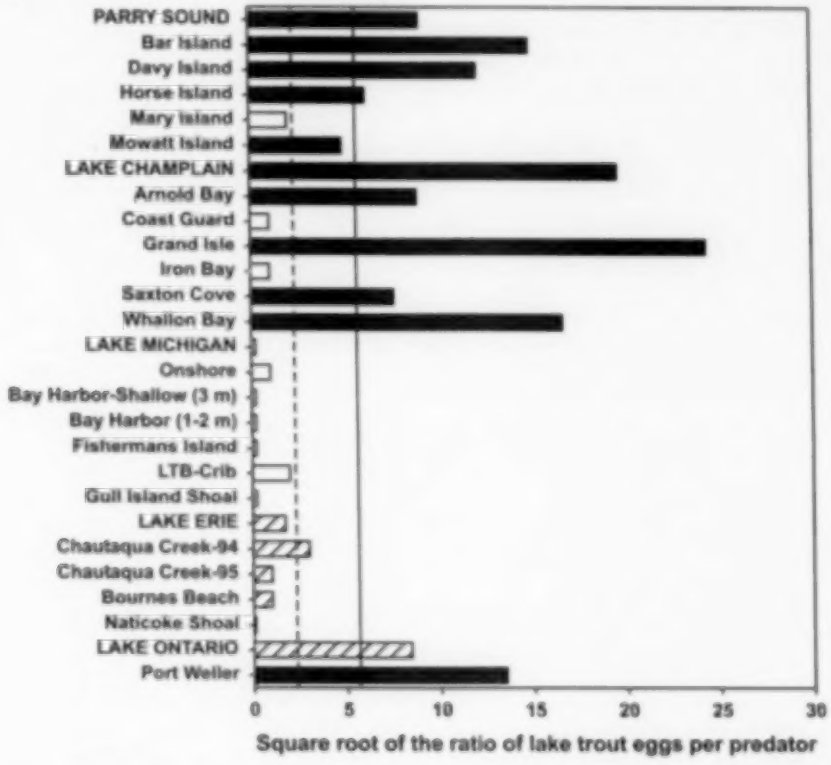
Fig. 5. Ratio of lake trout (*Salvelinus namaycush*) eggs deposited·predator^{-1}·m^{-2} at sites in northern Lake Michigan, Lake Champlain, and Parry Sound. Data from Lake Erie and Lake Ontario collected in previous investigations (J.D. Fitzsimons, personal communication) are also included. The broken line represents the largest ratio where fry emergence was not detected and the solid line represents the lowest ratio at sites with detectable fry emergence. Bars next to lake names represent the square root of average ratios for the region. To better represent small numbers, data were transformed to the square root of values. Solid bars indicate sites where emergence was detected, open bars indicate sites with no emergence, and hatched bars indicate sites where fry emergence was not assessed. LTB-Crib, Little Traverse Bay crib site.



surable recruitment that either there was insufficient spawning activity, high egg mortality, or some combination of the two. Lake Michigan may require higher numbers of spawning adults to recruit future year classes of lake trout.

Improved understanding of spawning habitat use and behaviors should prove to be useful to managers wishing to establish target egg densities. Our data indicate that, even in the face of abundant interstitial predators, it is possible to swamp a given area with enough eggs to allow for survival to the fry stage. Depensation likely occurs as more eggs are deposited per unit area and the available predators are satiated. Most predation occurs within the first 28 days after eggs are deposited, and eggs surviving this period are highly likely to survive through the winter period (Dittman et al. 1998; Claramunt et al. 2005). By concentrating spawning activity on certain areas, it may be easier to achieve target egg densities rather than focusing on regional or lake-wide abundance targets for adults.

Ratios of eggs per predator provide a simplistic way to index the effectiveness of lake trout rehabilitation efforts. In this case, the target density of eggs per predator is based on initial egg deposition minus the effect of predation and other losses prior to entering the winter period. The lowest number of eggs deposited per predator at locations that were successful in producing viable lake trout fry in our evaluations was 24 eggs·predator^{-1}·m^{-2}. Figure 5 represents a summary similar to that presented in Selgeby et al. (1995), using ratios instead of abundance to indicate reproductive potential. The average among the 10 successful spawning locations evaluated in this investigation was 190 eggs·predator^{-1}·m^{-2}; this may be a more reliable target for managers to ensure survival to the fry stage given additional density-independent sources of mortality and variation among systems. In Lake Michigan, where the highest egg-to-predator ratio was 4 eggs·predator^{-1}·m^{-2}, egg deposition would need to increase 20-fold to obtain egg survival similar to the average in Parry Sound (82 eggs·predator^{-1}·m^{-2}). Data from other investigations support these recommendations. Peck (1986) found egg-to-predator ratios range from 61–259 eggs·predator^{-1}·m^{-2} at sites with successful fry emergence in Presque Isle (Lake Superior). Work on Keuka Lake, New York, where lake trout are self-sustaining, has yielded ratios of 192 eggs·predator^{-1}·m^{-2} (Fitzsimons et al. 2005).

More detailed analyses of species assemblages and the size and density of predators at spawning sites provides further insight into the overall potential for predation effects

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(Horns and Magnuson 1981). The size of a predator directly influences the number of eggs consumed, while abundance levels, competition, and foraging behaviors likely strengthen or weaken this relationship in both conspecific and mixed-species environments (Gill and Hart 1996; Biga et al. 1998; Anderson 2001). Based on the potential for consumption of eggs, the hierarchy of predators observed in our study is fish > crayfish; round gobies > sculpin; and rusty crayfish > native crayfish. Exotic species in the latter two comparisons are more effective predators as they tend to be larger in size and tolerate dense populations of conspecifics.

Because crayfish dominated many of our spawning locations and fish dominated others, it is important for us to understand differences in foraging efficiency between fish and crayfish. The potential vulnerability of eggs may vary based on the type of predator and their physical position within rocky substrates. In studies comparing sculpin with *O. virilis*, predation rates were similar for the two species independently, but fish had a negative impact on crayfish foraging efficiency when species were together (Miller et al. 1992; J.D. Fitzsimons, G. Fodor, and B. Williston, unpublished data). Sculpin ate more eggs than *O. propinquus* in laboratory studies; however, when combined, crayfish negatively influenced sculpin populations by denying them access to refuge areas, indirectly increasing their vulnerability to predation (McNeely et al. 1990; Miller et al. 1992). In summary, crayfish were less efficient predators than sculpin, but likely influence the abundance of benthic fish populations through competition for habitat and are less vulnerable to predation (Olsen et al. 1991).

Crayfish were the most abundant predators in Parry Sound and Lake Michigan. *Orconectes rusticus* dominated the nearshore zone of northern Lake Michigan and *O. propinquus* dominated other locations in Lake Michigan and Parry Sound. Our work compliments other investigations by showing that, when both were present, the average size of *O. rusticus* was larger than that of *O. propinquus*, and that densities of *O. rusticus* were nearly twice as high (Olsen et al. 1991). *Cambarus robustus*, *O. limosis* and *O. virilis* were the largest of the five species of crayfish observed; however, the density and frequency of occurrence of these species was the lowest. Of the two predominant species of crayfish (*O. rusticus* and *O. propinquus*), *O. rusticus* would appear to be the more formidable predator; its higher density and larger size lead to higher potential consumption levels per individual when compared with the smaller *O. propinquus* and other large but less common species of native crayfish. Further, *O. rusticus* appears to be displacing *O. propinquus* when present, so populations are likely to increase in nearshore habitats throughout the Great Lakes.

Sculpin were the most abundant predator in Lake Champlain, and were the most common of the fish species collected in Parry Sound and Lake Michigan. In our investigation, the abundance of sculpin was lowest at sites with round goby populations when compared with sites without round gobies, suggesting that competitive displacement may be occurring since their arrival in northern Lake Michigan (Janssen and Jude 2001). In other investigations, round gobies have been found to interfere with breeding activities and compete for food and resources with sculpin (Dubs and Corkum 1996; Janssen and Jude 2001). In laboratory studies, round gobies were significantly more efficient at finding lake trout eggs at low densities (63 eggs·m^{-2}) than sculpin, and were found to eat three times the number of eggs (J.D. Fitzsimons, G. Fodor, and B. Williston, unpublished data). Round goby populations continue to increase (J.L. Jonas, C.M. Diana, and R.M. Claramunt, unpublished data), and like rusty crayfish, further growth and expansion of goby populations is likely. Based on their tendency toward higher population levels and greater individual search and egg consumption rates, round gobies are likely superior predators when compared with sculpins.

The abundance of mature lake trout occupying a given spawning location likely relates to the density of viable eggs deposited. To compare the relative abundance of adults among the three lakes, we have evaluated published accounts from other agencies (Reid et al. 2001; Bronte et al. 2003*b*; Elrott and Marsden 2004). Estimates of spawner abundance on sites in northern Lake Michigan were extremely low. In recent multi-agency fall assessment efforts, non-stocked offshore locations produced fewer than 25 fish·12 h^{-1}·305 m^{-1} gillnet, and nearshore locations produced between 35 and 50 lake trout·12 h^{-1}·305 m^{-1} gillnet (Bronte et al. 2003*a*). Estimates from Parry Sound, after adjusting catch per unit effort from lake trout·1.5 h^{-1}·91.7 m^{-1} gillnet to lake trout·12 h^{-1}·305 m^{-1} gillnet, were significantly higher than those observed for Lake Michigan, and ranged from 88 to 114 lake trout·12 h^{-1}·305 m^{-1} gillnet (Reid et al. 2001). Fall lake trout abundance estimates in Lake Champlain are not directly comparable with the other lakes, as lake trout were only assessed using electroshocking. In Lake Champlain, high egg deposition rates coincide with high spawner abundance levels primarily a result of stocking. Nearly twice the number of lake trout has been stocked per unit area in Lake Champlain (127 yearling equivalents·km^{-2}) when compared with Lake Michigan (72 yearling equivalents·km^{-2}). Parry Sound populations had been supplemented with stocked fish until 1997. Management actions increasing populations of spawning lake trout would likely elevate the number of eggs deposited per predator in a unit area.

The results of our investigation could be used to establish target densities of eggs deposited at a given location, and with further work, the target concentration of spawning adults needed to accomplish this. It is important to note that loss at the egg stage represents the first potential bottleneck to lake trout recruitment. We have not evaluated the influence of predation on post-swim-up fry, potentially the second bottleneck to lake trout recruitment.

Ecologists have hypothesized that predation may be the most important source of egg and larval mortality for fishes (Houde 2002). The mortality of early life stages due to predation represents a density-dependent response and is likely to decrease with increases in the numbers of lake trout eggs deposited and increase with higher abundances of predators. Other mortality factors may be density independent (i.e., thiamine deficiencies and physical disturbances) and can be responsible for significant losses at the egg and fry stage (Perkins and Krueger 1995; Fitzsimons et al. 1999). Density-dependent and independent sources of mortality should be considered in concert when determining the potential for

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survival of lake trout eggs and fry, because stock recruitment relationships require a measure of the reproductive potential of the population and the number of young that will reach maturity.

Acknowledgements

The Great Lakes Fishery Trust and the Great Lakes Fishery Commission provided funding for this project. We thank the captains and crew of the SV Steelhead, RV Shark, and RV Melosira. We thank the numerous staff, undergraduate, and graduate students who assisted with field and laboratory work, including the following: Eric Crissman, Anthony Kennedy, Brett Alger, and Dave Buintendorp of Michigan DNR; Mark Beekey, Jesse Wheeler, Eric Carlson, and Matt Toomey of UVM; and Bill Williston, Georgina Fodor, Gale Bravener, Cindy Chu, Kathy Seifried, and Silvina Carou of DFO. We also thank Bill Ridge for assistance with the rearing of lake trout at the Wellsboro USGS facility (USGS). We thank Patrick O'Neill, Adrienne Kral, and Steve Lenart of the Little Traverse Bay Bands of Odawa Indians as collaborators on the project. We thank Henk Don, Bruce Gray, Todd Breedon, and Dave Gilroy of the DOE Dive Team for their assistance in underwater field operations.

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Species separation and zoogeography of redfish and rockfish (genus *Sebastes*) by otolith shape analysis

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Abstract: Interspecific otolith shape variation was investigated within the species-rich genus *Sebastes* (redfish, rockfish) as a tool for species separation, since morphological distinction has proven to be difficult. Otolith samples from all four North Atlantic redfish species, six commercially important rockfish species from the North Pacific, and *Sebastes capensis* from the South Atlantic were compared for differences in linear otolith measurements and elliptical Fourier-shape descriptors. A clear distinction between the North Atlantic and North Pacific/South Atlantic species (97% correct jackknifed classification) was achieved by univariate measurements and discriminant analysis of size-corrected Fourier descriptors. Overall species classification success was 91%, 94% within the North Pacific species and 88% within the North Atlantic samples. From the Pacific rockfish, *Sebastes alutus* otoliths showed the strongest affinity to the North Atlantic *Sebastes*. High similarity of otolith shapes of *S. capensis* to the North Pacific *Sebastes* and clear discrimination from North Atlantic species coincide with current zoogeographical and speciation theories, as well as with recently reported genetic results.

Résumé : Nous avons évalué la variation interspécifique de la forme de l'otolithe comme outil d'identification dans le genre *Sebastes* (sébastes) qui contient de nombreuses espèces, parce que la distinction morphologique s'avère difficile. Nous avons comparé des échantillons d'otolithes des quatre espèces de sébastes (redfish) de l'Atlantique Nord, de six espèces de sébastes (rockfish) d'importance commerciale du Pacifique Nord et de *Sebastes capensis* de l'Atlantique Sud à la recherche de différences dans les mesures linéaires des otolithes et les descripteurs de Fourier de forme elliptique. Des mesures unidimensionnelles et une analyse discriminante des descripteurs de Fourier corrigés en fonction de la taille indiquent une distinction nette entre les espèces de l'Atlantique Nord et celles du Pacifique Nord et de l'Atlantique Sud (classification par la méthode d'Eustache correcte à 97 %). Le succès global de la classification est de 91 %, soit 94 % pour les espèces du Pacifique Nord et 88 % pour les échantillons de l'Atlantique Nord. Chez les sébastes du Pacifique, ce sont les otolithes de *Sebastes alutus* qui montrent le plus d'affinité pour ceux des *Sebastes* de l'Atlantique Nord. La forte similarité de la forme des otolithes de *S. capensis* avec celles des *Sebastes* du Pacifique Nord et leur distinction nette d'avec celles des espèces de l'Atlantique Nord s'accordent bien avec les théories actuelles sur la zoogéographie et la spéciation et aussi avec les données génétiques récentes.

[Traduit par la Rédaction]

Introduction

The genus *Sebastes* Cuvier includes the highest number of species within the family Scorpaenidae, with over 100 species worldwide, most of them occurring in the North Pacific. Along the North Pacific coasts, the commonly termed rockfish species range from Kyushu/Japan and Taiwan in the west over the Bering Sea and the Alaskan coast to Baja California and the Gulf of California in the east (Love et al. 2002). Following an antitropical distribution, *Sebastes* in the Southern Hemisphere is limited to two closely related species. *Sebastes oculatus* is found from the Pacific coast of Peru and Chile (Kong Urbina 1985) east to the Falkland Islands, marking the western distribution boundary of *Sebastes capensis*, which expands eastwards to the Tristan da Cunha group and South Africa (Eschmeyer 1969). While southern California represents the hot spot of *Sebastes* species diversity in the North Pacific with up to 60 species (Love et al. 2002), the North Atlantic is inhabited by only four species, *Sebastes fasciatus* (being limited to the Northwest Atlantic), *Sebastes viviparus* (limited to the Northeast Atlantic), *Sebastes marinus*, and *Sebastes mentella*, commonly known as redfish (Whitehead et al. 1986).

Attempts at taxonomic division of the genus *Sebastes* have been numerous and subject to large revisions (Kendall 2000). Various biochemical and genetic studies have been

Received 2 August 2004. Accepted 19 April 2005. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on 21 September 2005.
J18247

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 doi: 10.1139/F05-143 © 2005 NRC Canada

Table 1. Summary of otolith sampling information from *Sebastes* species in the Atlantic and Pacific.

Species	Species code	Region	Area	Sampling period	Mean fish length (and range) (cm)	No. of otoliths
S. fasciatus	fas	North Atlantic	Southern Newfoundland	April 1997	32.9 (26–43)	37
S. marinus	mar	North Atlantic	Flemish Cap, Greenland, Iceland, Barents Sea	July 2000 – July 2001	40.0 (36–57)	117
S. mentella	men	North Atlantic	Flemish Cap, Davis Strait, Greenland, Iceland, Irminger Sea, Faroe Islands, Barents Sea	October 1998 – October 2001	38.9 (34–49)	593
S. viviparus	viv	North Atlantic	Barents Sea	July–August 2000	21.0 (19–25)	20
S. alutus	alu	North Pacific	British Columbia	October 1977 – October 1978	40.9 (36–49)	94
S. brevispinis	bre	North Pacific	British Columbia	March–December 1978	53.0 (44–65)	106
S. entomelas	ent	North Pacific	British Columbia	July–September 1978	51.7 (40–59)	111
S. flavidus	fla	North Pacific	British Columbia	June 1977 – August 1978	48.0 (41–55)	64
S. proriger	pro	North Pacific	British Columbia	May–October 1978	36.3 (31–42)	46
S. reedi	ree	North Pacific	British Columbia	August–November 1978	42.3 (38–47)	38
S. capensis	cap	South Atlantic	Tristan da Cunha, South Africa	February–July 2002	29.3 (26–36)	39
Total						1265

Note: Fish length is given as fork length. Only mature fish were selected, and only otoliths from one fish side were taken for analysis.

employed since the 1960s to resolve inter- and intra-specific relationships. Investigations on haemoglobin patterns (Tsuyuki et al. 1968; Nedreaas et al. 1994) and protein electrophoresis (Johnson et al. 1972) were followed by more advanced techniques using mtDNA (Bentzen et al. 1998) and more specifically the cytochrome *b* gene of the mtDNA control region (Johns and Avise 1998; Rocha-Olivares et al. 1999*a*, 1999*b*) as well as microsatellite variation (Roques et al. 1999). Also, fatty acid analyses were evaluated for use as species-specific markers of Atlantic *Sebastes* (Joensen and Grahl-Nielsen 2000).

The traditional approach to species identification involves morphometric and meristic characters of the fish body and was predominantly applied to the morphologically very similar Atlantic redfish (Power and Ni 1985; Valentin et al. 2002). Morphometric analyses on body hard parts such as otoliths, however, are less affected by short-term changes in fish condition, stress, or starvation (Campana and Casselman 1993) but could be influenced by environmental factors (Cardinale et al. 2004). Descriptions of otolith morphology have been used to investigate evolutionary aspects and phylogeny of various fish species to identify fossil discoveries (Nolf 1995), as well as fish remains in stomachs of marine mammals or seabirds (Fitch and Brownell 1968). More recently, linear otolith morphometrics and otolith shape analysis (Lombarte and Castellón 1991; Gauldie and Crampton 2002) were adopted for species discrimination, allowing quantification of interspecific differences.

Rasmussen (1958) was the first to describe differences in otolith morphology of Atlantic redfish. Species-characteristic otolith shapes are documented for most North Atlantic fish (Harkonen 1986; Campana 2004), but quantification of the observed differences by otolith morphometrics has been rare. Since the redfish species in the North Atlantic show high similarity between species and large intraspecific variation regarding morphological and genetic features (Nedreaas et al. 1994), the otolith shapes of Atlantic redfish were investigated as a tool for species identification. By comparing Atlantic and Pacific *Sebastes* species, the overall variation within the genus *Sebastes* was evaluated as a companion study to concurrently investigated intraspecific variation in otolith morphology and inferred stock discrimination of the widely distributed and migrating Atlantic redfish species *S. marinus* and *S. mentella* (Stransky 2005). To achieve these goals, univariate shape indices and elliptical Fourier descriptors (FDs) were analysed for differences between all four North Atlantic redfish species, six commercially important North Pacific *Sebastes* species, and *S. capensis* from the South Atlantic. Current knowledge on zoogeographical relationships within this genus and speciation theories are discussed in relation to the quantified interspecies variation in otolith shapes.

Material and methods

Sampling

Sagittal otoliths were taken from fish collected on research vessels or from commercial catches using bottom trawl gears, with the exception of *S. mentella* taken in the Irminger Sea with a pelagic Gloria-type gear. Otolith samples from the four North Atlantic *Sebastes* species (redfish) were obtained from research cruises in typical fishing areas of the respective species between April 1997 and October 2001, aiming at a complete coverage of the distribution area (Table 1; Fig. 1). Sampling periods of several weeks or months were necessary in some cases to achieve this coverage. Although *S. viviparus* is known to occur off East Greenland and Iceland and around the Faroe Islands, the number of otoliths available from these areas was too small to be added to the Norway/Barents Sea sample. Species separation during sampling was carried out by external identification features, whereas samples from *S. fasciatus* and *S. mentella* in the Northwest Atlantic were discriminated by differences in the gas bladder musculature (Ni 1981). The

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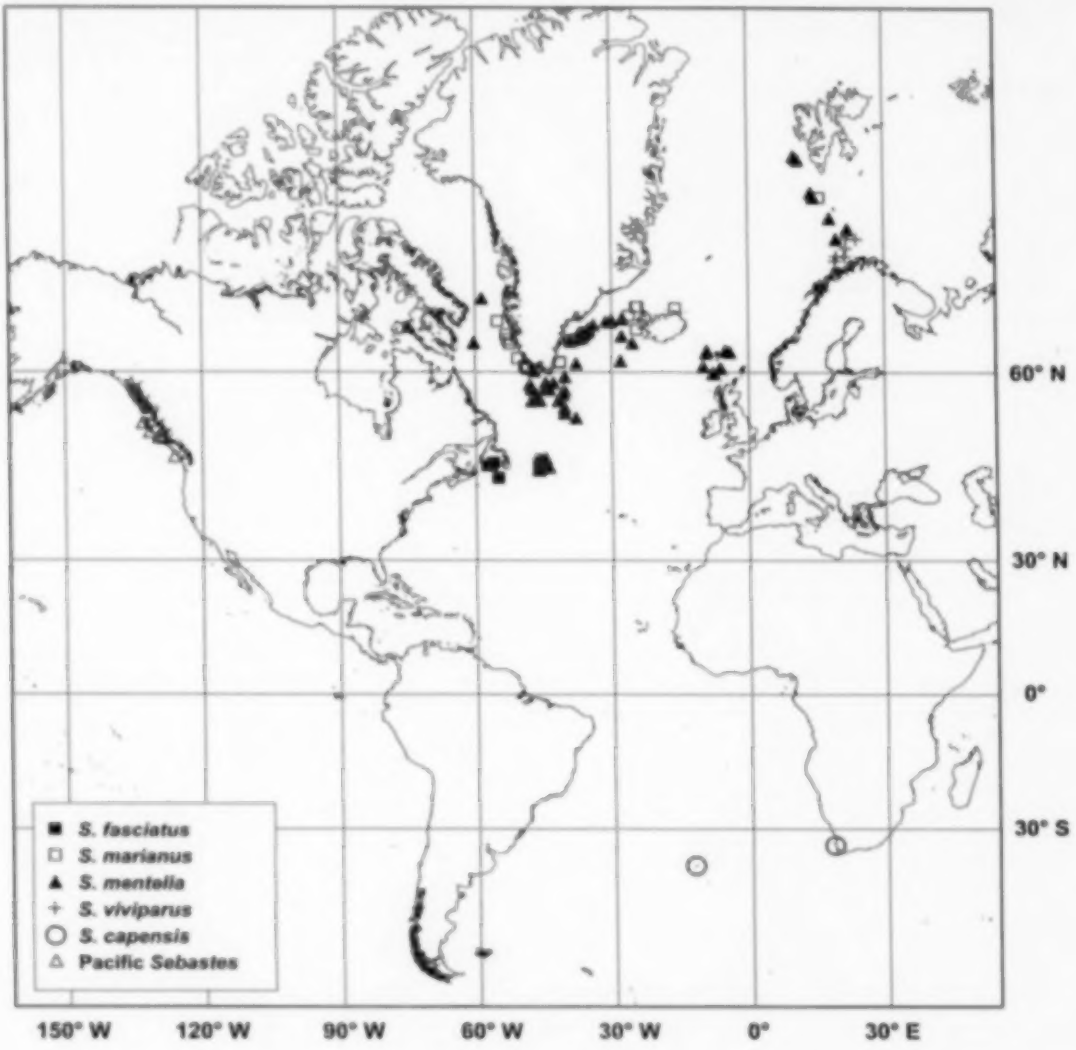
Fig. 1. Sampling positions of *Sebastes* otoliths from the North Atlantic (redfish: *S. fasciatus*, *S. marinus*, *S. mentella*, and *S. viviparus*; separately marked), North Pacific (rockfish: *S. alutus*, *S. brevispinis*, *S. entomelas*, *S. flavidus*, *S. proriger*, and *S. reedi*), and South Atlantic (*S. capensis*).



otolith samples were taken as pairs and stored dry in paper envelopes including individual fish data (e.g., length, weight, and sex). The North Pacific samples were taken from six commercially important *Sebastes* species (rockfish) landed by commercial trawlers operating off British Columbia between June 1977 and December 1978 (Table 1; Fig. 1). Since the exact positions of the trawls were not available, the midpoints of the areas where the vessels operated (groundfish areas 3C, 5A, 5B, 5D, and 5E; see Rutherford 1999) were drawn into the sampling map (Fig. 1). These single otolith samples were stored in glass tubes filled with glycerine for conservation, with otoliths of a certain fork length centimetre class being separated from the neighbouring centimetre class by paper separators. In the South Atlantic, otoliths of *S. capensis* were collected on fishing trips off Gough Island (Tristan da Cunha) and near Cape Town/South Africa in February and July 2002, respectively, and stored in paper envelopes.

Only otoliths from sexually mature fish were included in the data analysis to minimize variability caused by size effects and to standardize the selected fish length range to a common life history landmark. Length-at-maturity (L_{50}) data for the respective species and areas available from the literature were used to determine the minimum fish length at which the corresponding otoliths could be included. Average L_{50} values reported by Ni and Sandeman (1984) and St-Pierre and de Lafontaine (1995) were used for *S. fasciatus*, *S. marinus*, and *S. mentella* in the Northwest Atlantic, while the corresponding values for *S. marinus*, *S. mentella*, and *S. viviparus* in the Northeast Atlantic and Arctic were taken

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from Trout (1961), Sorokin et al. (1986), and Magnússon and Magnússon (1995). When more than one estimate for the same species in the same area was available, the maximum value was selected. Since female redfish generally become mature with fish lengths 1–2 cm larger than the males, the data sets were divided by sex to account for differences in maturation. For the North Pacific *Sebastes* species off British Columbia, L_{50} data were provided by sex by Haldorson and Love (1991). No L_{50} data were reported for *S. capensis*, but regular investigations off South Africa indicated length-at-maturity of around 26 cm (T. Andrew, Enviro-Fish Africa Ltd., Department of Ichthyology and Fisheries Science, Rhodes University, P.O. Box 94, Grahamstown, South Africa, personal communication). For most of the species, only fork length data were recorded. Thus, total length data provided for *S. marinus*, *S. mentella*, and *S. viviparus* were converted to fork length based on available regressions (F. Saborido-Rey, Institute of Marine Research, Eduardo Cabello 6, 36208 Vigo, Spain, unpublished data).

The selected otoliths were further restricted to the left body side to ensure that one sample represents one fish and to avoid possible asymmetric effects. Trial comparisons of shape descriptors derived from left otoliths and mathematically mirrored right otoliths, however, revealed nonsignificant discriminant analysis results (Wilks' $\lambda > 0.8$, $p > 0.05$). The reduced data set included a total of 1265 otoliths (North Atlantic: $n = 767$, North Pacific: $n = 459$, South Atlantic: $n = 39$) (Table 1).

Image and shape analysis

Otolith outlines were digitized using an image analysis system consisting of a high-resolution monochrome CCD video camera mounted on an Olympus® microscope and connected to a PC framegrabber card via BNC video cable. The microscope magnification was adjusted to the size of the otoliths to ensure as high resolution as possible, varying between 30× and 50×. The image analysis system was calibrated in the horizontal and vertical directions separately to avoid possible distortion effects of the lens system. The otoliths were positioned onto a microscope slide with the sulcus down and the rostrum to the left in horizontal line to minimize distortion errors within the normalization process. High-contrast video images were produced using transmitted light, delivering dark two-dimensional objects with a bright background. The video signal was analysed using Optimas® 6.51 image analysis software (Media Cybernetics 1999). Shape digitization was performed by sampling 1000 equidistant points on each outline, representing the resolution of the video camera. For the export of outline coordinates, Optimas® macros were applied.

A set of univariate descriptors was calculated based on the digitized x,y coordinates. In addition to otolith length (major axis length), otolith breadth (minor axis length), and area, two shape descriptors, circularity and rectangularity, were recorded. Circularity is a dimensionless value defined as the area perimeter squared divided by the area, resulting in a minimum of 4π (~12.57) that is obtained from boundaries describing a circle (Media Cybernetics 1999). Rectangularity is a dimensionless number between 0 and 1 defined as the object's area divided by the area of the enclosing box orientated along the major axis. The rectangularity of a square is 0.5, whereas a circle reaches $\pi/4$ (~0.79), and a long and narrow rectangle has a value of 1. After removal of adhering tissue and blood remains from the otolith surface and a minimum of 24 h of air-drying at room temperature, all otoliths were weighed with a precision of 0.1 mg. Differences in univariate descriptors between species and regions were tested by analysis of variance (ANOVA).

The digitized outline coordinates were forwarded to elliptical Fourier analysis Kuhl and Giardina 1982) using C++ modules based on the algorithms of Ferson et al. (1985). The principle methodology of Fourier analysis has been described elsewhere (e.g., Lestrel 1997) and will therefore not be presented here. The elliptical Fourier analysis represents a fitting of harmonic functions to the original otolith outlines with an ellipse as the first approximation step. The algorithm for normalizing the rotation and starting angle of the outline was modified to account for deviations from the horizontal axis resulting from the positioning of the otolith on the microscope slide. During the elliptical Fourier analysis, the size, location, and starting point of the object outlines within the two-dimensional space were normalized. Only the first 17 harmonics were included in the statistical analysis, since these were responsible for over 99% of the shape variation (Lestrel 1997). This selection results in 65 FDs for each sample consisting of 3 × 16 FDs (harmonics 2–17 each) of the sine and cosine parts of the x direction and sine part of the y direction (where FDs of harmonic 1 become constants after the normalization process, see above) plus 17 FDs (harmonics 1–17) of the cosine part of the y direction. Before analysing the FDs for differences between species, the distribution of these data was tested. All FD amplitudes were normally distributed (Kolmogorov–Smirnov test of normality, $p > 0.05$). Thus, no transformation of the FD data was necessary.

Size correction and multivariate analysis

Most important for morphometric analysis is the correction of the data by size, i.e., uncoupling of otolith shape and size in this case. This was accounted for by using the residuals of the common within-group slopes (Reist 1985) of the linear regressions of each FD on otolith length. Otolith length was chosen as the size factor to be removed from the variables because fish length for the North Pacific species was found to be very variable within similar otolith length classes owing to possible misinterpretation or mixing of fish length interval layers in the otolith storage tubes. The FD residuals were compared between groups (species or regions) using linear discriminant function analysis (Klecka 1980). The classification success into groups was tested by jackknifed cross-validation (SPSS Inc. 1999). Differences between sexes and temporal effects (for areas where more than one sampling interval was carried out) were also tested by discriminant analysis. Average otolith shapes were drawn for each species from reproduced outlines of the average FDs within a species.

Results

Univariate shape descriptors

Clear differences in the otolith length – otolith breadth relationships between regions (North Atlantic, North Pacific, and South Atlantic) and species were observed (Fig. 2) and

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Fig. 2. Relationship between otolith length (OL) and otolith breadth (OB) for all *Sebastes* species. Linear regressions were fitted through all individuals belonging to the same region (North Atlantic: OB = 0.420 OL + 2.484, r^2 = 0.734; North Pacific: OB = 0.311 OL + 2.522, r^2 = 0.446; South Atlantic: OB = 0.463 OL–0.061, r^2 = 0.714).

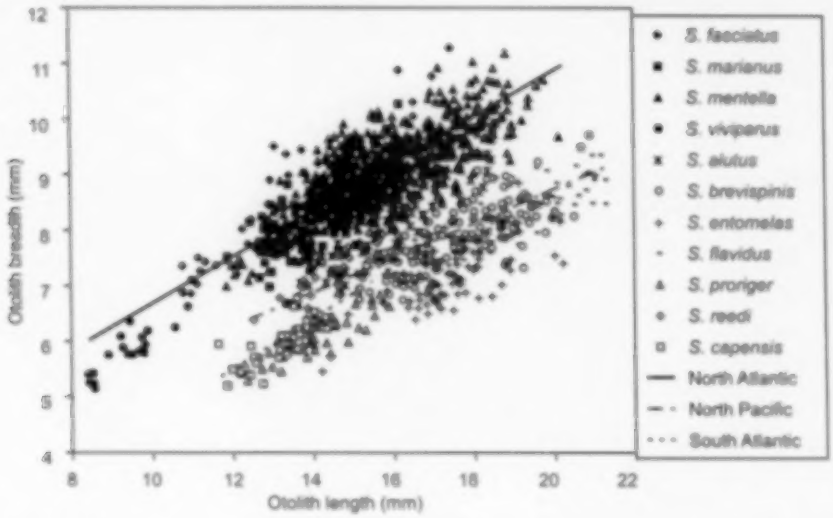
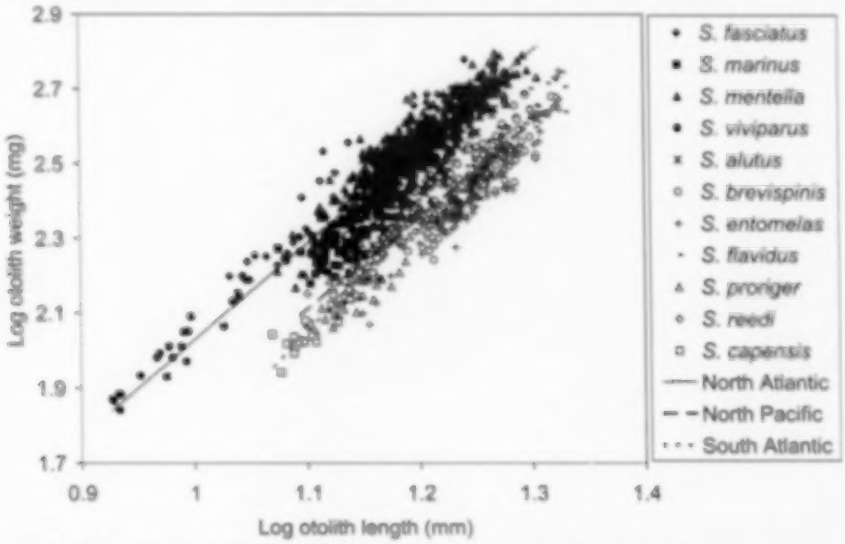


Fig. 3. Relationship between otolith length (OL) and otolith weight (OW) for all *Sebastes* species. Linear regressions were fitted through all individuals belonging to the same region (North Atlantic: log OW = 2.576 log OL – 0.547, r^2 = 0.875; North Pacific: log OW = 2.501 log OL – 0.644, r^2 = 0.757; South Atlantic: log OW = 3.067 log OL – 1.322, r^2 = 0.842).



confirmed by ANOVA of the otolith breadth to otolith length ratios (main effect regions: $F_{[2,1262]}$ = 1516.84, p < 0.0001; species: $F_{[10,1254]}$ = 571.63, p < 0.0001). Otoliths of the North Atlantic species were considerably broader than otoliths of the North Pacific and South Atlantic species, with most of the species exhibiting distinctive within-region differences and *S. alutus* samples showing the greatest overlap with the North Atlantic species. The *S. capensis* samples had an otolith length – otolith breadth relationship close to that of the North Pacific samples.

A similar separation of North Atlantic and North Pacific/South Atlantic species was obvious from the log-transformed otolith length – otolith weight regressions (Fig. 3), with a clearer differentiation within the North Atlantic species. Especially the *S. marinus* samples appeared to be separated from *S. mentella*, showing the strongest overlaps with *S. alutus*. The log otolith weight to log otolith length ratios indicated highly significant differences between regions (ANOVA, $F_{[2,1261]}$ = 1022.37, p < 0.0001) and species (ANOVA, $F_{[10,1253]}$ = 507.78, p < 0.0001).

The variation of the shape descriptors circularity and rectangularity generally increased with increasing otolith length (Figs. 4 and 5). While a considerable overall trend of decreasing circularity (as given by higher values) with in-

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Fig. 4. Relationship between otolith length (OL) and circularity (Circ.) for North Atlantic (squares), North Pacific (circles), and South Atlantic (triangles) *Sebastes* species. Linear regressions were fitted through all individuals belonging to the same region (North Atlantic: Circ. = 0.487 OL + 14.178, r^2 = 0.234; North Pacific: Circ. = 1.024 OL + 7.975, r^2 = 0.309; South Atlantic: Circ. = 1.247 OL + 6.229, r^2 = 0.297).

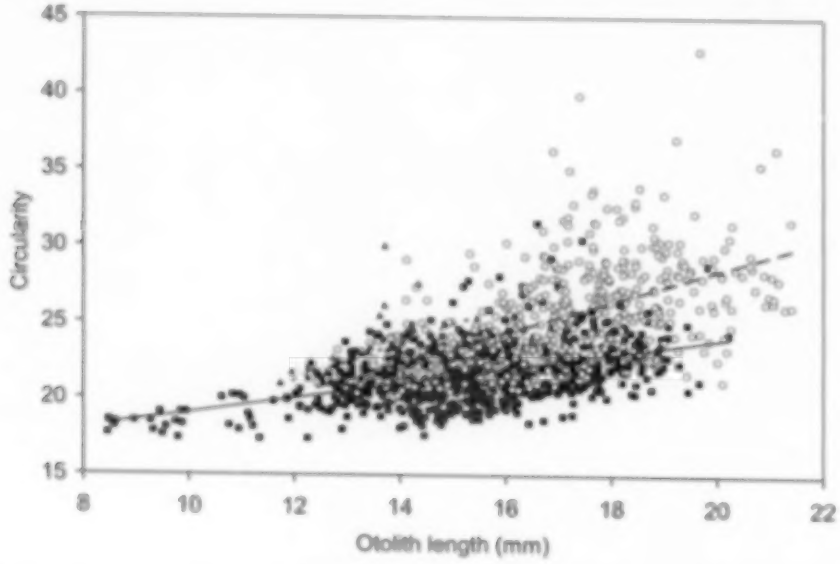
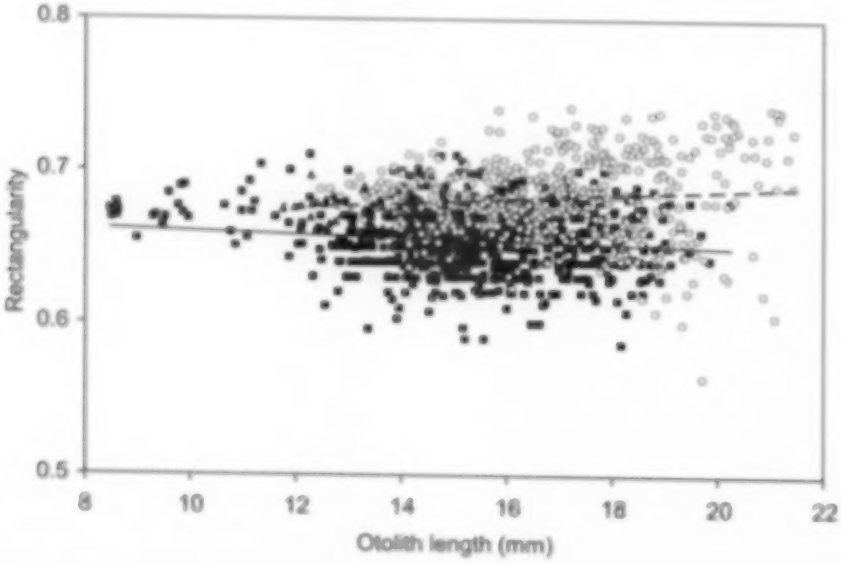


Fig. 5. Relationship between otolith length (OL) and rectangularity (Rect.) for North Atlantic (squares), North Pacific (circles), and South Atlantic (triangles) *Sebastes* species. Linear regressions were fitted through all individuals belonging to the same region (North Atlantic: Rect. = –0.001 OL + 0.670, r^2 = 0.009; North Pacific: Rect. = 0.002 OL + 0.658, r^2 = 0.011; South Atlantic: Rect. = –0.002 OL + 0.6939, r^2 = 0.011).



creasing otolith length was apparent (Fig. 4), no clear trend could be derived from the rectangularity plot (Fig. 5). Otoliths of the North Atlantic species, however, were characterized by higher circularity (lower values) and lower rectangularity than otoliths of the North Pacific and South Atlantic species (ANOVA, circularity: $F_{[2,1262]}$ = 303.59, p < 0.0001; rectangularity: $F_{[2,1262]}$ = 230.39, p < 0.0001).

Fourier shape analysis

The size correction of the FDs by residuals reduced the correlation between FDs and otolith lengths very effectively. Pearson correlation before size adjustment was up to r = 0.49 with more than two thirds of the FDs being significantly correlated (p < 0.05) with otolith length, while the residuals showed no correlation with otolith length (r < 6 ×

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Fig. 6. Discriminant function scores for the Fourier descriptors of otoliths from *Sebastes* species in the Atlantic Ocean and Pacific Ocean. The first discriminant axis captures 55.8% of the variation; the second axis captures 15.2%. North Atlantic species are marked with solid symbols and appear on the left side of the plot, and North Pacific and South Atlantic species, clustered on the right side of the plot, are marked with open symbols, asterisks, crosses, and dashes.

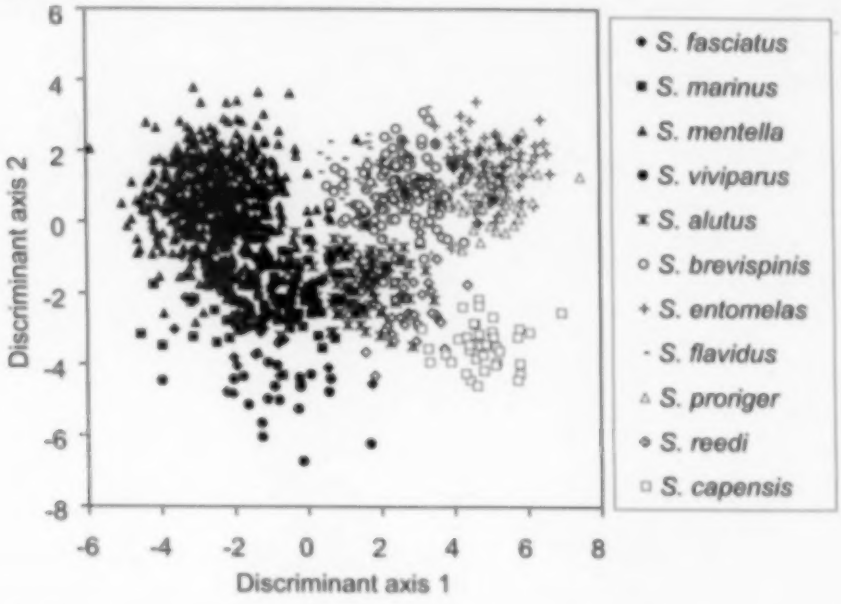

Table 2. Jackknifed classification matrix of the discriminant analysis between *Sebastes* species.

	fas	mar	men	viv	alu	bre	ent	fla	pro	ree	cap
fas	**75.7** (28)	2.7 (1)	18.9 (7)	0 (0)	2.7 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
mar	7.7 (9)	**82.9** (97)	5.1 (6)	0 (0)	2.6 (3)	0.9 (1)	0 (0)	0.9 (1)	0 (0)	0 (0)	0 (0)
men	2.4 (14)	4.4 (26)	**91.4** (542)	0.2 (1)	0.7 (4)	0.3 (2)	0 (0)	0.3 (2)	0 (0)	0.2 (1)	0.2 (1)
viv	0 (0)	0 (0)	0 (0)	**100.0** (20)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
alu	2.1 (2)	2.1 (2)	1.1 (1)	0 (0)	**83.0** (78)	1.1 (1)	2.1 (2)	4.3 (4)	0 (0)	4.3 (4)	0 (0)
bre	0 (0)	0.9 (1)	0 (0)	0 (0)	0 (0)	**89.6** (95)	3.8 (4)	1.9 (2)	3.8 (4)	0 (0)	0 (0)
ent	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	**90.1** (100)	2.7 (3)	7.2 (8)	0 (0)	0 (0)
fla	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1.6 (1)	0 (0)	**98.4** (63)	0 (0)	0 (0)	0 (0)
pro	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	**100.0** (46)	0 (0)	0 (0)
ree	0 (0)	0 (0)	0 (0)	0 (0)	5.3 (2)	0 (0)	0 (0)	0 (0)	0 (0)	**94.7** (36)	0 (0)
cap	0 (0)	2.6 (1)	0 (0)	0 (0)	2.6 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	**94.9** (37)

Note: The percentages in rows represent the classification into the areas given in columns; the corresponding number of individuals is given in parentheses. The percentages of correctly classified species are in bold. Overall classification success is 91.0% (Wilks' $\lambda = 0.0016$). See Table 1 for definition of species codes.

10^{-14}, $p > 0.05$). No significant differences were found between sexes (Wilks' $\lambda = 0.92$, $p > 0.05$).

Discriminant analysis based on the size-corrected FD data of all analysed species revealed a North Atlantic and a North Pacific/South Atlantic cluster with relatively small overlap (Fig. 6). *Sebastes viviparus* and *S. capensis* appear as considerably well-separated groups in the lower part of each cluster. Overall jackknifed species classification success was 91%, ranging from 76% correct classification for *S. fasciatus* to 100% for *S. proriger* and *S. viviparus* (Table 2). Differences between species were highly significant (Wilks' $\lambda = 0.0016$, $p < 0.001$). Individual fish could be correctly classified into regions by 97% (Table 3), with the South Atlantic (*S. capensis*) being best discriminated from the other regions.

Species differentiation within regions was further investigated by separate analyses for the North Atlantic and North Pacific. The discriminant scores plot for the North Atlantic species (Fig. 7) illustrates a considerable overlap of *S. marinus* and *S. mentella* otolith shapes, with the *S. fasciatus* samples showing high variability and *S. viviparus* being well discriminated from the other species. The North Atlantic species were classified with 88% accuracy (Table 4), ranging from 81% (*S. fasciatus*) to 100% (*S. viviparus*). The highest misclassification rates were found for *S. mentella*, 16% of the *S. fasciatus* samples and 9% of the *S. marinus* samples being allocated to this species. Within the North Pacific, overall classification success into species (94%) (Table 5) was markedly higher than for the

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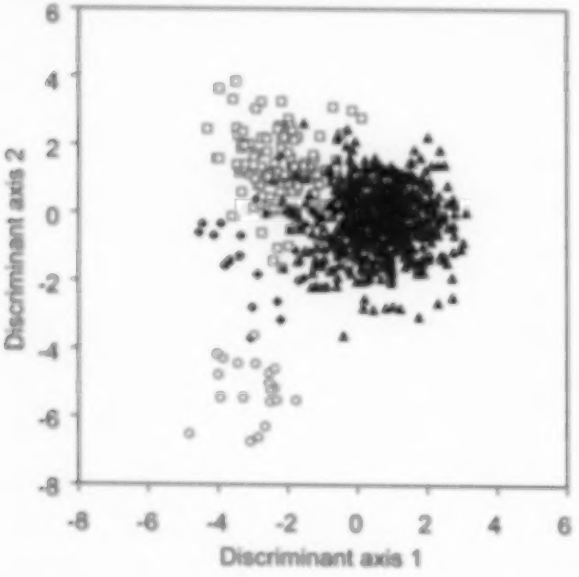
Table 3. Jackknifed classification matrix of the discriminant analysis between regions (see Table 1 for region allocations).

	North Atlantic	North Pacific	South Atlantic
North Atlantic	**97.0** (744)	2.6 (20)	0.4 (3)
North Pacific	2.2 (10)	**96.5** (443)	1.3 (6)
South Atlantic	0 (0)	2.6 (1)	**97.4** (38)

Note: The percentages in rows represent the classification into the areas given in columns; the corresponding number of individuals is given in parentheses. The percentages of correctly classified regions are in bold. Overall classification success is 97.0% (Wilks' $\lambda = 0.1156$).

Fig. 7. Discriminant function scores for the Fourier descriptors of otoliths from *Sebastes* species in the North Atlantic, *S. fasciatus* (diamonds), *S. marinus* (squares), *S. mentella* (triangles), and *S. viviparus* (circles). The first discriminant axis captures 49.9% of the variation, while the second axis captures 31.6%.



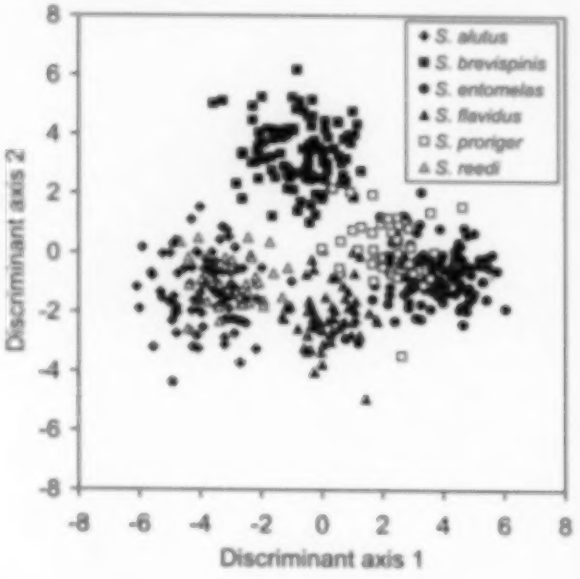
North Atlantic. *Sebastes entomelas* otoliths were misclassified by 11% into the *S. proriger* group, representing the largest observed overlap with this region (Fig. 8). One hundred percent correct classification was achieved for *S. flavidus*.

The average otolith shapes by species, scaled to the same size, showed distinct differences between North Atlantic and North Pacific *Sebastes*, with considerable variation within these regions (Fig. 9). Otoliths of North Atlantic redfish are generally broader than Pacific *Sebastes* otoliths and have a marked rostrum indentation (upper left part of the outlines in Fig. 9). *Sebastes capensis* otoliths were very similar to the North Pacific otoliths.

Discussion

This study demonstrates clear differences in otolith shapes of North Atlantic and North Pacific *Sebastes* species and interspecific variation in univariate measurements and indices as well as multivariate shape descriptors.

Fig. 8. Discriminant function scores for the Fourier descriptors of otoliths from *Sebastes* species in the North Pacific. The first discriminant axis captures 50.7% of the variation, while the second axis captures 24.5%.



Univariate shape descriptors

Two major species groups were determined by univariate measurements. Higher otolith breadth and otolith weight in relation to otolith length discriminate North Atlantic redfish from the North Pacific *Sebastes* and *S. capensis*. Differences in allometric otolith growth in length and breadth (Simoneau et al. 2000) as well as thickness, with otolith weight as a proxy for three-dimensional growth, are responsible for the observed differentiation. Representing the traditional and predominantly followed approach to otolith shape variation owing to relatively straightforward data acquisition, differences in univariate (linear) otolith morphometrics have been used to discriminate fish species and stocks (e.g., Lombarte and Castellón 1991).

Univariate shape indices calculated as a combination of otolith perimeter and area, such as circularity and rectangularity, have been used to evaluate otolith shape variation in other fish species (Bolles and Begg 2000). In this study, North Pacific *Sebastes* generally showed higher circularity and rectangularity than North Atlantic *Sebastes*, with considerable more overlap between regions. The common trend of increasing shape complexity and variation with larger fish or otolith sizes was also supported in this study, indicated by higher circularity (high otolith perimeter in relation to otolith area) and increased variation in rectangularity for larger otoliths. The reason for an increased otolith perimeter with otolith size is a higher number of indentations and variations on the otolith outline as the fish grow older. While there are approaches to utilize this feature for fish ageing (Doering and Ludwig 1990), otolith complexity could be related to life history effects, such as ear-dependent predation (Aguirre and Lombarte 1999) that gains relevance in greater depths (Gauldie and Crampton 2002).

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Table 4. Jackknifed classification matrix of the discriminant analysis between North Atlantic *Sebastes* species.

	S. fasciatus	*S. marinus*	*S. mentella*	*S. viviparus*
S. fasciatus	**81.1** (30)	2.7 (1)	16.2 (6)	0 (0)
S. marinus	7.7 (9)	**82.9** (97)	8.5 (10)	0.9 (1)
S. mentella	3.0 (18)	7.4 (44)	**89.2** (529)	0.3 (2)
S. viviparus	0 (0)	0 (0)	0 (0)	**100.0** (20)

Note: The percentages in rows represent the classification into the species given in columns; the corresponding number of individuals is given in parentheses. The percentages of correctly classified species are in bold. Overall classification success is 88.3% (Wilks' $\lambda = 0.1302$).

Table 5. Jackknifed classification matrix of the discriminant analysis between North Pacific *Sebastes* species.

	S. alutus	*S. brevispinis*	*S. entomelas*	*S. flavidus*	*S. proriger*	*S. reedi*
S. alutus	**91.5** (86)	1.1 (1)	0 (0)	4.3 (4)	0 (0)	3.2 (3)
S. brevispinis	0.9 (1)	**94.3** (100)	0.9 (1)	0 (0)	3.8 (4)	0 (0)
S. entomelas	0 (0)	0 (0)	**87.4** (97)	1.8 (2)	10.8 (12)	0 (0)
S. flavidus	0 (0)	0 (0)	0 (0)	**100.0** (64)	0 (0)	0 (0)
S. proriger	0 (0)	2.2 (1)	4.3 (2)	0 (0)	**93.5** (43)	0 (0)
S. reedi	2.6 (1)	0 (0)	0 (0)	0 (0)	0 (0)	**97.4** (37)

Note: The percentages in rows represent the classification into the species given in columns; the corresponding number of individuals is given in parentheses. The percentages of correctly classified species are in bold. Overall classification success is 94.0% (Wilks' $\lambda = 0.0023$).

Fig. 9. Average shapes of *Sebastes* otoliths from the North Atlantic (thick solid lines; *S. fasciatus*, *S. marinus*, *S. mentella*, and *S. viviparus*), North Pacific (broken lines; *S. alutus*, *S. brevispinis*, *S. entomelas*, *S. flavidus*, *S. proriger*, and *S. reedi*), and South Atlantic (thin solid line; *S. capensis*).

Fourier and multivariate analysis

A complete description of otolith shape can only be accomplished by an outline technique that captures the entire shape variation and small-scale individual differences on the otolith outline. Fourier series analysis has proven to be the most powerful technique (Campana and Casselman 1993), although the biological interpretation of these data is more complex than for linear morphometrics. The elliptical Fourier analysis used in this study appeared to be the most appropriate method for otolith outlines, since otolith shape can be best described by an ellipse (Chauvelon and Bach 1993), and thus, approximation to original shapes is achieved by fewer steps than in conventional Fourier transformation. Based on the interspecific differences in elliptic Fourier amplitudes, individual relationships between and within *Sebastes* species could be investigated and quantified in this study. The separation of the North Atlantic and North Pacific/South Atlantic species groups observed in the univariate data was supported by 97% classification success into regions using the multivariate FD data. The *Sebastes* otoliths investigated in this study could be allocated correctly to species by over 90%, the North Atlantic species exhibiting lower classification success than the North Pacific species owing to relatively low discrimination of *S. fasciatus* from *S. mentella* and *S. marinus*. These species are known to be closely related, as expressed by similar body morphometrics (Power and Ni 1985; Valentin et al. 2002) and low interspecific genetic variation (Nedreaas et al. 1994; Bentzen et al. 1998). In the Northwest Atlantic, the discrimination of *S. fasciatus* from *S. mentella* by morphometric characters has been difficult, and in the Northeast Atlantic, *S. marinus* and *S. mentella* are hard to distinguish from each other in some areas. In our study, these species pairs separated with 81%–89% and 83%–89% classification, respectively. Classification success for the North Atlantic species, however, did not improve significantly by leaving out sampling areas where intermediate types of *S. marinus* and *S. mentella* are found regularly, such as East Greenland (Nedreaas et al. 1994).

As the final step of the multivariate shape analysis of *Sebastes* otoliths, the reconstruction of the average shapes by species, based on the FDs that explained over 99% of shape variation, highlighted major differences between North Atlantic and North Pacific samples and a strong similarity of *S. capensis* to the North Pacific *Sebastes*. This overlay technique also identified the breadth dimension and antirostrum outline parts as being responsible for the observed differences between regions and interspecific shape variation. Albeit the advantage of a direct comparison of shapes, similar studies presenting average shapes (Lombarte and Castellón 1991; Campana and Casselman 1993) have not utilized this technique.

Confounding effects

Intraspecific variation of otolith shapes of those species with the highest number of samples taken over a large geo-

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graphic area, i.e., *S. marinus* and *S. mentella*, was considerably higher than for all other species. As only a fraction of the distribution area of the Pacific *Sebastes* species was covered, the impact of geographic sampling variability on our results could not be investigated fully. This could only be addressed by sampling otoliths of these species from their entire distributional range. Since the otolith samples from the selected North Pacific rockfish were taken in the late 1970s, over 20 years before the North Atlantic and *S. capensis* samples were collected, temporal effects could confound the presented results. Comparisons of FD data for *S. mentella* from East Greenland (where two sampling intervals were available), however, revealed no significant differences between sampling years. On an evolutionary scale, the time lag in collection dates between North Pacific and Atlantic samples is negligible. The related effect of different age groups or year-classes present in similar fish or otolith length groups could not be directly tested owing to missing ageing data for the investigated otoliths. Trial multivariate analyses of age-corrected FDs derived from available age–length keys (R. Stanley, Fisheries and Oceans Canada, Pacific Biological Station, 3190 Hammond Bay Road, Nanaimo, BC V9T 6N7, Canada, personal communication; F. Saborido-Rey, Institute of Marine Research, Eduardo Cabello 6, 36208 Vigo, Spain, unpublished data) showed species differences that were not significantly different from the presented patterns. On the other hand, uncertainty and high variation in ageing data for North Atlantic redfish (Nedreaas 1990; Stransky et al. 2005) and North Pacific *Sebastes* species (e.g., Kimura et al. 1979) could have introduced additional error. The comparison of otolith length – otolith weight relationships allows limited evaluation of age effects, since otolith weight can be employed as a proxy for age (Pawson 1990). Otolith weight related to otolith length of *S. mentella* was higher than for *S. marinus*, caused by a higher number of additional growth layers in proximal direction (thickness) with higher longevity. This can also be observed for several rockfish species (MacLellan 1997). The impact of environmental differences on growth rates and related effects on otolith shape (Campana and Casselman 1993; Cardinale et al. 2004) could not be tested directly in this study. However, a comparison of reported growth rates of the investigated *Sebastes* species (Love et al. 2002) with the discriminant functions of this study did not reveal a causal relationship.

Zoogeography and speciation

Unambiguous differentiation of the investigated *Sebastes* otolith shapes into a North Atlantic and a North Pacific group as well as the affinity of *S. capensis* otoliths to North Pacific species support current theories on the speciation of the genus *Sebastes*. The Northwest Pacific appears to be the origin and centre of distribution for the genus *Sebastes* (Eschmeyer 1969), having spread to the north and east with the expansion of cold water about 10–13 million years ago (Kendall 1991). The four North Atlantic *Sebastes* species have most probably originated from transarctic movement of one common ancestor species (Barsukov 1981). The observed similarity of *S. alutus* otolith shapes with the North Atlantic *Sebastes*, especially with *S. marinus* in the univariate comparisons, is in accordance with Kendall (1991) who found that larvae of *S. alutus* have an appearance similar to that of larvae of the North Atlantic species. There are also similarities between *S. alutus* and *S. marinus* in life history parameters such as fecundity (Halldorson and Love 1991). Deep climatic changes in the Tertiary had greater influence in the Atlantic than in the Pacific (Briggs 1970), enabling higher radiation rates in the Pacific and thus differentiation of the numerous Pacific *Sebastes* species. Pacific rockfish have then occupied a large variety of different habitats, ranging from shallow kelp beds and rocky areas and reefs to depths exceeding 500 m (Matthews 1990; Yoklavich et al. 2000), whereas Atlantic redfish are mainly restricted to deeper demersal or pelagic habitats. Some bathymetric separation is apparent in the North Atlantic, *S. mentella* being distributed deeper than *S. fasciatus*, *S. marinus*, and *S. viviparus*. The variation in otolith shapes within the North Atlantic, however, was not much different to North Pacific *Sebastes*, as would have been expected from evolutionary aspects and genetic variation (Rocha-Olivares et al. 1999*a*).

Otolith shapes of *S. capensis* from the South Atlantic showed strong similarities with the North Pacific samples, coinciding with the zoogeographical considerations of Eschmeyer and Hureau (1971) and genetic analyses of Rocha-Olivares et al. (1999*b*). During cooling and then rewarming of equatorial waters, ancestors of the present Southern Hemisphere *Sebastes* likely have crossed the tropics of the eastern Pacific and speciated into at least two sibling species. *Sebastes oculatus* inhabited the coasts off Chile, Peru, and the Falkland Islands, from where *S. capensis* speciated and moved eastwards via West Wind drift to Tristan da Cunha and South Africa (Rocha-Olivares et al. 1999*b*). In the Atlantic, however, a transequatorial movement of *Sebastes* from north to south is unlikely, since invasion of *S. capensis* into the South Atlantic probably took place before or during the North Atlantic species evolved. This theory was supported by Rocha-Olivares et al. (1999*a*) who compared the cytochrome *b* sequence of 54 *Sebastes* species and found distinct differences between *S. marinus* and *S. capensis*. Confirmation of these results on a phenotypic level was achieved for the first time in this study, showing clear separation of *S. capensis* otolith shapes from the North Atlantic *Sebastes* samples.

Although otoliths from all four North Atlantic species, from six typical North Pacific species, and *S. capensis* from the South Atlantic were investigated in this study, full evaluation of the variability in otolith morphometry within the genus *Sebastes* will only be possible by a complete coverage of all known species. This would require an extensive sampling programme that will probably take several years but would enable comprehensive morphometric analysis of this incredibly diverse genus.

Acknowledgements

We would like to express our gratitude to Don Power (Fisheries and Oceans Canada, St. Johns, Newfoundland), Kjell Nedreaas (Institute of Marine Research, Bergen, Norway), Jakúp Reinert (Faroese Fisheries Laboratory, Tórshavn,

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Faroe Islands), Fran Saborido-Rey (Institute of Marine Research, Vigo, Spain), Thorsteinn Sigurdsson (Marine Research Institute, Reykjavík, Iceland), and Margaret Treble (Fisheries and Oceans Canada, Winnipeg, Manitoba) for providing redfish otoliths from all over the North Atlantic, Rick Stanley (Fisheries and Oceans Canada, Nanaimo, British Columbia) for giving permission to use the Pacific samples for this study and for providing sample and biological data, and James Glass (Natural Resources, Tristan da Cunha) and Rob Leslie and Chris Wilke (Marine and Coastal Management, Cape Town, South Africa) for collecting *S. capensis* otoliths from distant places in the South Atlantic. Thanks to all staff members and volunteers taking part in the sampling, Jürgen Schlickeisen for assistance with OPTIMAS and programming, and Cornelius Hammer, Rick Stanley, and three anonymous referees for helpful comments on the manuscript. This work was partly funded by the European Commission (R&D project REDFISH, QLK5-CT1999-01222). During the preparation of the image and shape analysis methodology, the first author benefitted from a doctoral grant of the German National Academic Foundation (Studienstiftung des deutschen Volkes).

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Using tree-ring crossdating techniques to validate annual growth increments in long-lived fishes

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Abstract: We applied crossdating, a dendrochronology (tree-ring analysis) age validation technique, to growth increment widths of 50 *Sebastes diploproa* otoliths ranging from 30 to 84 years in age. Synchronous growth patterns were matched by the following: (*i*) checking the dates of conspicuously narrow growth increments for agreement among samples and (*ii*) statistically verifying that growth patterns correlated among samples. To statistically verify pattern matching, we fit each time series of otolith measurements with a spline, and all measurements were divided by the values predicted by the curve. This standardized each time series to a mean of 1, removing the effects of age on growth and homogenizing variance. Each time series was then correlated with the average growth patterns of all other series, yielding an average correlation coefficient (r) of 0.53. Average growth of all 50 samples was significantly correlated with an upwelling index ($r = 0.40$, $p = 0.002$), the Pacific Decadal Oscillation ($r = -0.29$, $p = 0.007$), and the Northern Oscillation Index ($r = 0.51$, $p = 0.0001$), corroborating accuracy. We believe this approach to age validation will be applicable to a wide range of long-lived marine and freshwater species.

Résumé : Nous avons utilisé la datation croisée, une technique de validation de l'âge en dendrochronologie (analyse des anneaux des arbres), pour étudier les largeurs des incréments de croissance des otolithes de 50 *Sebastes diploproa*, âgés de 30 à 84 ans. Les patrons de croissance synchronisée ont été corroborés (*i*) en nous assurant que les dates d'incréments particulièrement étroits correspondent dans les échantillons et (*ii*) en vérifiant statistiquement que les patrons de croissance sont en corrélation dans les différents échantillons. Pour vérifier statistiquement la correspondance des patrons, chaque série chronologique de mesures d'otolithes est ajustée à une spline et toutes les mesures sont divisées par les valeurs prédites par la courbe. Cette opération standardise chaque série chronologique autour d'une moyenne de 1, éliminant les effets de l'âge sur la croissance et rendant la variance homogène. Chaque série chronologique est alors mise en corrélation avec le patron de croissance moyen des autres séries, ce qui génère un coefficient de corrélation (r) moyen de 0,53. La croissance moyenne de l'ensemble des 50 échantillons est en corrélation significative avec l'indice d'affleurement (upwelling) ($r = 0,40$, $p = 0,002$), l'oscillation décennale du Pacifique ($r = -0,29$, $p = 0,007$) et l'indice d'oscillation boréale ($r = 0,51$, $p = 0,0001$), ce qui confirme sa précision. Nous croyons que cette méthode de validation de l'âge pourra s'appliquer à une variété d'espèces de mer et d'eau douce à grande longévité.

[Traduit par la Rédaction]

Introduction

Counts of annual growth increments formed in otoliths provide a relatively quick, convenient method for estimating fish age. Age is estimated from hundreds of thousands of otoliths each year, with a minimum of over 1 million fish aged either by scales or otoliths in 1999 alone (Campana and Thorrold 2001). These data are of critical importance in the assessment of fish stocks, as well as in estimates of growth and mortality rates. Yet despite their importance in fisheries management, significant errors may occur (Boehlert and Yoklavich 1984). Complications may arise from the asymmetric form of the otolith and the tendency of numbers and widths of increments to vary among different regions of the otolith. Also, boundaries of the increments may be diffuse and difficult to identify. Consequently, considerable error may occur in the interpretation of otoliths, and age estimates may vary among readers and laboratories (Beamish 1979; Boehlert and Yoklavich 1984). In most cases, ageing errors depart from the actual age of the fish by 1 or 2 years, but more extreme errors are not uncommon, especially in longer-lived fish (Wilson and Boehlert 1990). Management policies based on inaccurate data could lead to the overexploitation of fish populations.

A number of age-validation techniques have been developed to reduce this error, several of which only roughly estimate the lifespan of long-lived individuals. One of the most common of these methods is bomb radiocarbon, which uses as a time-specific marker the sudden increase of ^{14}C in the marine environment that occurred following nuclear testing in the 1950s and 1960s (Kalish 1993; Campana et al. 2001). Levels of ^{14}C in otolith cores should correspond to time-

Received 30 July 2004. Accepted 12 April 2005. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on 17 September 2005.
J18243

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 doi: 10.1139/F05-142 © 2005 NRC Canada

specific increases in marine ^{14}C. In comparison with known ^{14}C chronologies for the region, over-ageing would shift the otolith ^{14}C chronology to earlier years and under-ageing would shift the otolith ^{14}C chronology to later years (Kalish 1993; Campana et al. 2001). Thus, ageing errors can be corrected by aligning otolith ^{14}C with marine ^{14}C values. In a related technique, decay rates of radioisotopes are used as a chronometer for estimating otolith age. Half-lives serve as an indicator of time elapsed since the radioisotopes were incorporated into the otolith. A frequently applied technique for older fish is calculating the ratio of ^{210}Pb to ^{226}Ra in the core of the otolith, which is useful for time scales up to approximately 120 years (Bennett et al. 1982; Burton et al. 1999; Andrews et al. 2002). Though radioisotopes and bomb carbon are used to confirm longevity of very old fish, they lack the annual resolution necessary to validate an exact age or exact number of growth increments.

On a finer scale, other techniques have been developed to precisely validate the annual nature of ring formation and assign the correct calendar year to each growth increment. Most of these techniques require an event at a known date that imparts a unique, identifiable mark on a bony structure of the fish. To date, these studies have largely used chemical or isotopic signatures in bony structures, particularly otoliths. For example, one of the most rigorous approaches is the release and recapture of marked fish of a known age. At the time of release, the fish may be injected with a compound such as oxytetracycline, which distinctly fluoresces when exposed to ultraviolet light. When the fish is recaptured, the number of otolith increments is checked against the time elapsed since release (Beamish and Chilton 1982; Leaman and Nagtegaal 1987; Heifetz et al. 1998). Presently, mark–recapture studies are considered to be the most precise and accurate means of age validation (Beamish and McFarlane 1983; Campana 2001). Yet they are best applied over relatively short periods because the probability of recapturing a fish declines over time.

Though much less thoroughly explored than the use of chemical and isotopic signatures for age validation, morphological variations within an otolith could also be used as time-specific markers for age validation. Growth increment width integrates many potential time-specific markers. It reflects the overall physiological condition of the fish, which in turn is a function of competition, access to food, and the physiological and developmental status (age, size, vigor, phase in life cycle) of the fish over time. More importantly, otolith growth can reflect conditions of the physical environment, such as sea surface temperature, or other physical factors affecting prey availability or physiological condition. Indeed, strong El Niño events, such as that which occurred in 1983, appear to have reduced the growth increment width in yellowtail and widow rockfish (Woodbury 1999). Also, Pacific whiting, splitnose rockfish, and canary rockfish increment-width chronologies were significantly influenced by a variety of physical variables, including sea surface temperature and upwelling (Boehlert et al. 1989; Dorn 1992). In freshwater systems, ring-width chronologies of calcified growth structures were significantly correlated with climatic variables, as demonstrated for the freshwater drum in Minnesota, rock bass in streams of the Ozark Mountains, and lake sturgeon in the Great Lakes region (Guyette and Rabeni 1995; Pereira et al. 1995; LeBreton and Beamish 2000). Finally, thermally induced marks on otoliths of young fish produce "bar codes" that can later be used to estimate fish age (Volk et al. 1994). In recommending appropriate validation techniques in fish ageing, Beamish and McFarlane (1983, p. 741) suggested that "It may be possible to find some 'natural' mark on a structure that identifies a particular year class, but such marks appear to be very rare." However, these natural markers may be much more common than originally thought.

It is this correspondence between growth increment and physical environment that we use as the basis for dating each annual increment, and ultimately, for age validation. We apply a technique from dendrochronology (tree-ring analysis) called crossdating, which is used to cross-match growth patterns among samples to assign the correct calendar year to each growth increment. Data sets with properly dated growth increments can be used to generate high-resolution growth chronologies, as well as to determine the exact age of each tree. Crossdating is based on the assumptions that growth increments form at regular intervals (in this case, annually), growth increment width is limited by some aspect of climate, and the intesity of this climatic variable fluctuates over time. If these assumptions hold true in marine systems, climate will induce synchronous, time-specific growth patterns in otolith increment widths, much as it does in tree rings. To ensure that each growth increment is properly dated, growth patterns are visually matched by checking that the calendar years of conspicuously narrow or wide growth increments tend to correspond among samples. For statistical verification, climate-induced growth patterns are isolated from each time series and cross-correlated with the growth patterns of other specimens. High correlations indicate a high degree of precision among the otolith time series, while low correlations suggest ageing errors. We then correlate otolith growth patterns with climate records to corroborate accuracy, thus verifying that the correct calendar year is assigned to each growth increment. If otolith growth patterns correlate strongly among fish and with records of the physical environment, each growth increment should be assigned the correct calendar year and age estimates of each fish should therefore be correct.

Crossdating among samples is considered the most important step in any tree-ring analysis and serves as the principle on which modern dendrochronology was founded (Stokes and Smiley 1996). Assigning the correct calendar year to all growth increments is imperative if tree rings are to be used to date archaeological ruins or reliably reconstruct climate, forest dynamics, or forest age structure. Despite the parallels between the two fields, very few sclerochronology (analysis of growth increments in hard structures) studies have attempted to apply dendrochronology to otolith age validation and development of increment-width chronologies (Guyette and Rabeni 1995; Pereira et al. 1995; LeBreton and Beamish 2000). Thus, in this study we demonstrate that crossdating is possible for validating the ages of long-lived splitnose rockfish (*Sebastes diploproa*). This species serves as an example of a large number of rockfish species that live to ages in excess of 50 and even 100 years, to which this technique will likely apply (Munk 2001). The annual precision and accuracy afforded from crossdating could provide valuable age

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data for fishery management and complement the suite of age validation techniques already in use.

Methods

Sebastes diploproa otoliths were collected during rockfish surveys conducted by the Alaska Fisheries Science Center (NOAA Fisheries, Seattle, Washington, USA) in 1989 and 1995. All fish were collected from late June through mid-August off the west coast of the United States between 35°N and 40°N. At this time of year, *Sebastes* have typically completed a hyaline zone and have begun formation of an opaque zone with the onset of the new growing season (Pearson 1996; Woodbury 1999). Otoliths were embedded in resin and mounted on a diamond lapidary saw; thin sections approximately 0.4 mm in thickness were cut along a dorsal–ventral axis of the otolith. Cuts were perpendicular to the sulcus and passed through the focus of each otolith. Thin sections were then mounted on glass slides and polished by hand using 400- and 2000-grit sandpaper. Otolith age was preliminarily determined by counting annual growth increments along one continuous axis from the distal dorsal surface to the proximal dorsal surface. One increment consisted of an opaque summer growth zone and a translucent winter growth zone. All otoliths less than 30 years of age were omitted from further analysis so there would be an adequate number of degrees of freedom for subsequent correlation analyses. From the set of specimens at least 30 years in age, otoliths were selected in which increment borders were crisp and readily identifiable.

Crossdating was accomplished with the following two-step process: (*i*) a visual check of the dates of narrow and wide growth increments for general agreement among samples and (*ii*) statistical verification by cross-correlating growth increment measurements among samples. We began with the clearest otolith thin sections in which all growth increments were easily identified. Starting at the otolith margin, we proceeded toward the focus, noting the dates of conspicuously narrow or wide growth increments. This direction was opposite to that of most ageing procedures, which typically proceed from the focus to the margin. Yet this reversal was necessary so that we could tentatively date each growth increment by counting back from the partial increment formed at the margin during the year of capture. Growth increments were noted as conspicuously narrow (or wide) if they appeared to be noticeably reduced (or increased) in comparison with the immediate one to three neighbors on both sides. Many of these conspicuous growth increments, referred to by dendrochronologists as signature years, should be synchronous among samples (Yamaguchi 1991; Stokes and Smiley 1996). As we evaluated the otoliths, we found that the dates of many conspicuously narrow or wide growth increments corresponded among samples. These signature years occurred, on average, once per decade. For each otolith, we checked that the dates of conspicuously narrow or wide growth increments agreed with those in other samples. If a growth increment had been missed, tentative dates of narrow or wide increments would be shifted by a year, and the point at which the shifting began indicated the location of the error. Variability did occur among samples, yet the weight of evidence suggested all otoliths were properly aligned. We were careful not to impose changes in the calendar years of growth increments without first clearly identifying the missed or falsely added increment.

Measurement of the otolith growth increments was conducted using the program ImagePro Plus v. 5.0 (Media Cybernetics, Silver Spring, Maryland). Images were captured with a Leica DC300 7.2 megapixel digital camera attached a Leica $MZ9_5$ dissection scope with maximum magnification of 75×. Increments were measured from the dorsal proximal edge of the otolith to the focus, or as close to the focus as possible (Fig. 1). The axis of measurement was limited to radii in which all growth increments could be clearly discerned. Consequently, the location of the axis of measurement varied somewhat among otoliths. For consistency, our axis of measurement followed the axis of growth, which in most cases was a straight line. If the axis of growth curved, a curved measurement axis was used to ensure that all measurements followed a course parallel to the axis of growth (i.e., perpendicular to the axis of the increment boundaries). In some specimens, the boundaries of the youngest or oldest few increments were not clear enough to allow for measurement. For these otoliths, all other growth increments were measured, and we used the calendar years assigned to each increment by simple counts or visual crossdating.

Statistical validation of crossdating was performed by isolating the common climate signal from the time series of otolith measurements, and then cross-correlating these growth patterns among all samples. The first step in this process was to isolate climate-induced growth patterns by partitioning them from all other influences on the width of otolith growth increments. Other variables affecting increment width included age, competition, the physiological and developmental status of each fish, and any artifacts of otolith preparation. In tree rings, climatic effects can be distinguished from other influences by isolating high-frequency variability. Although climate varies on a range of time scales and causes long-term variations in growth increment, climate is unique in that it induces high-frequency (year-to-year) variation in growth increments (Fritts 1976). Indeed, this concept is the basis of using signature years to visually crossdate time series: extreme climate events less than a year in duration yield a conspicuous growth increment. In contrast, age and competition tend to create much more sustained variations in widths of annual growth increments. In trees, such age-related and other long-term trends are removed by fitting each time series of measurements with a mathematical function, and then dividing the observed measurements by the expected values predicted by that function. Negative exponential, power, or linear functions are most frequently used. Where more flexibility is necessary, a cubic spline may better fit the measurements. We applied all these functions to otolith measurements and chose the ones that yielded the highest R^2 value, or in the case of the spline, provided a much better fit upon visual inspection of the function superimposed on the measurements. Removal of the age-related trend was then accomplished by dividing measurements by expected values predicted from the best-fit function. However, after this process of detrending, time series may still contain autocorrelation that could mask the high-frequency variability used for crossdating or induce

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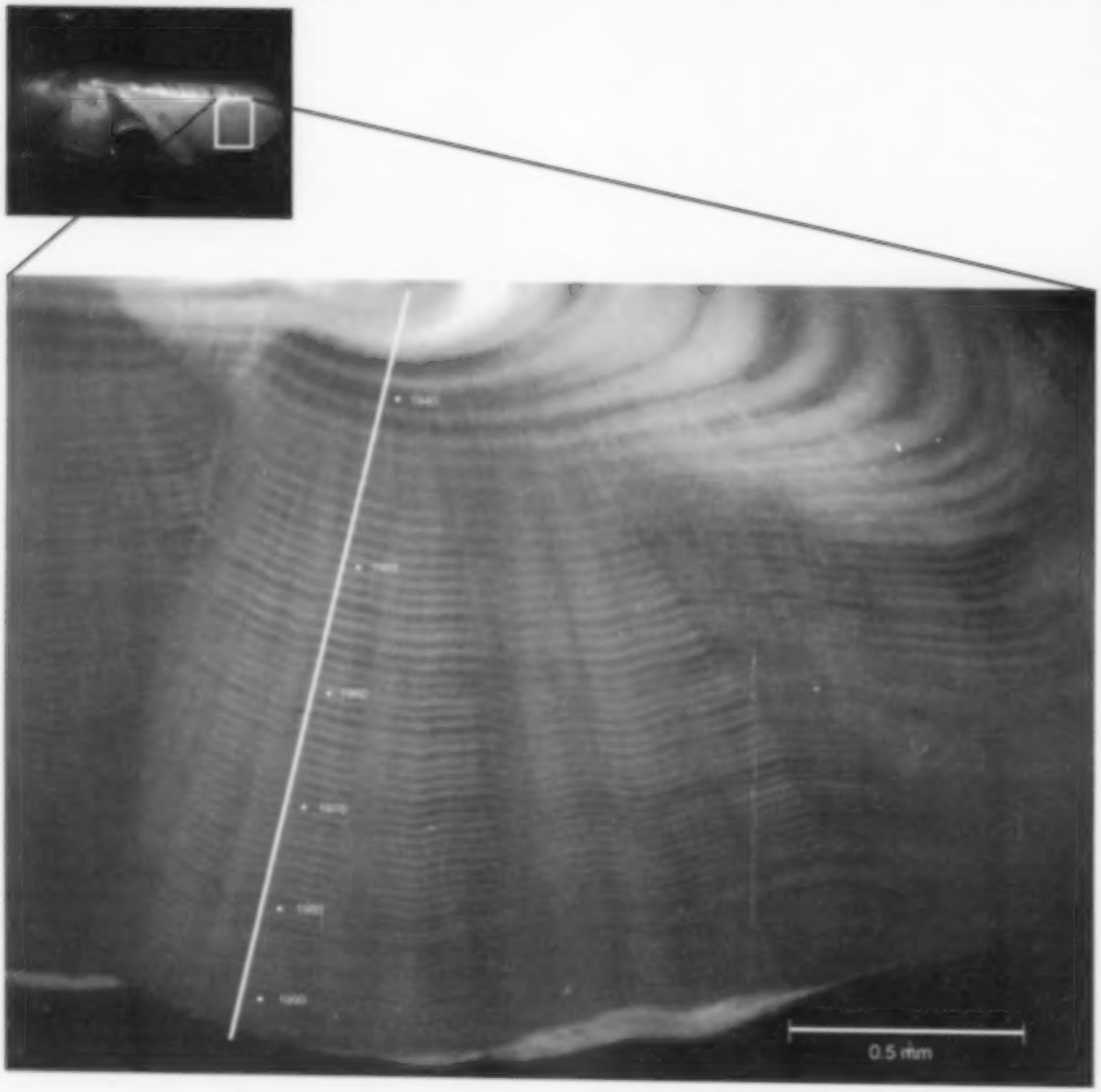
Fig. 1. Magnification of *Sebastes diploproa* otolith cross section. Annual growth increments are dated with white dots and the axis of measurement is indicated by a white line.



spuriously high correlations among samples. Any remaining long-term trends were therefore eliminated by removing as much autocorrelation (persistence) as possible from each detrended otolith time series using the autoregressive modeling. The resulting residual time series should contain almost exclusively high-frequency variability.

Once the climate signal was isolated by detrending and removing any remaining autocorrelation, crossdating was statistically validated by cross-correlating all residual otolith time series. In this final step, residual otolith time series were averaged to form a master chronology of sample-wide growth. Then each residual otolith time series was correlated with the master chronology. Yet before each correlation was performed, the otolith time series was removed from the master chronology to avoid comparing the tested series against itself. Correlations were performed 50 times; once for each residual otolith time series and the master chronology containing the average of all other otolith time series. Residual otolith time series were broken into 50-year segments that overlapped by 25 years (i.e., 1925–1975, 1950–2000), and each segment was correlated with the master chronology. Splitting each chronology into segments helped identify whether errors occurred early or late in the time series. The program COFECHA was used to automate all

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phases of statistical crossdating. COFECHA was written in 1982 by Richard Holmes of the Laboratory of Tree-Ring research at the University of Arizona, and is available through the International Tree-Ring Data Bank Program Library (ITRDBL) at the University of Arizona Laboratory of Tree-Ring Research website (http://www.ltrr.arizona.edu/software.html) (Holmes 1983; Grissino-Mayer 2001). A final splitnose rockfish chronology was compiled by averaging all 50 residual chronologies into a master chronology.

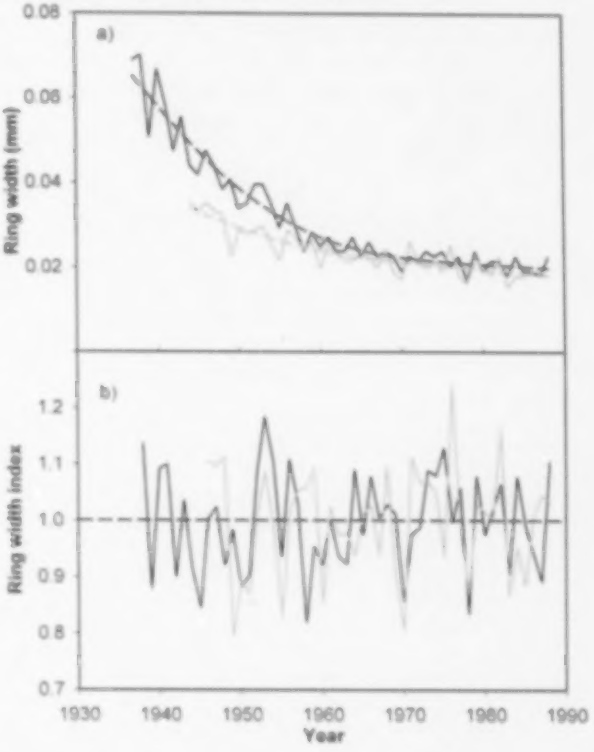
We correlated three climatic variables with the resulting splitnose rockfish biochronology: the Northern Oscillation Index (NOI), the Pacific Decadal Oscillation (PDO), and an upwelling index. The NOI and upwelling index were obtained through the Pacific Fisheries Environmental Laboratory website (http://www.pfeg.noaa.gov/). The upwelling index is calculated as wind stress divided by Coriolis parameter, in which positive values indicate upwelling and negative values indicate downwelling (PFEL 2004). NOI is the difference in sea level pressure anomalies between the North Pacific High (at 35°N, 135°W) and Darwin, Australia. The NOI provides a direct link to the North Pacific, unlike the more traditionally used Southern Oscillation Index (SOI), which calculates pressure differences between Darwin, Australia and Tahiti. Negative values of the NOI imply El Niño events, while positive values imply La Niña events (PFEL 2004). PDO values were obtained from the NOAA (National Oceanographic and Atmospheric Administration) and the University of Washington Joint Institute for the Study of the Atmosphere and the Ocean website (http://www.jisao.washington.edu/pdo/). Positive PDO values indicate warm phases, while negative values indicate cool PDO phases (Mantua et al. 1997).

Results and discussion

Signature years were evident in the growth increments of the splitnose otoliths. Narrow signature years included 1983, 1978, 1954, 1936, and to a lesser extent, 1988, 1970, and 1958, while 1971 was consistently wide. Not every signature year occurred in each otolith, and the prominence of the signature years varied among samples. Yet all otoliths did exhibit some combination of these signature years, which allowed for crossdating. The strongest example of a signature year was the 1983 increment, which was consistently narrow in almost all samples. Signature years crossdated in most otoliths on the first attempt of counting back from the outermost increment. In some cases, an increment would be missed, and the tentative dates of signature years offset. However, these unclear growth increments could be readily discerned on closer inspection. We did not reassign calendar years to growth increments without clearly identifying the initial error. In no case did we discover a year in which a fish did not form an increment, even in 1983. The same signature years were common to fish of all ages, and among otoliths collected in 1989 and 1995.

As in tree-ring measurements, the width of a growth increment decreased as the fish aged (Fig. 2*a*). This age-related decline is due to the changing geometry of the otolith and asymptotic growth of the fish. Though simple age-related declines occurred in the otolith time series, negative linear, negative exponential, or power functions did not fit these data. Cubic splines fit the decreasing increment-width trends much more closely, so we chose these functions to detrend otolith measurements (Fig. 2). As an additional step to this analysis, we attempted to identify the ideal spline rigidity necessary to maximize the power of crossdating in this data set. Spline rigidity can range from following every third growth increment at its most flexible to a straight line at its most rigid. Rigidity of a spline is typically expressed in terms of the wavelength at which 50% of the variance is removed. The shorter the set wavelength, the more flexible the spline will be. In dendrochronological applications, crossdating is typically optimized with a spline rigidity of 32 years (Holmes 1983). For otoliths, we began with a 32-year spline, and then experimented with a number of rigidity values. The optimum rigidity will be the one in which the average correlation among standardized series is the highest, which indicates that undesirable signals have been removed, and the common, sample-wide signal is maximized. After testing rigidities from 10 to 70 years, we found that 22 years yielded the highest average correlations among each otolith time series and the master chronology. Thus, we applied a spline with a rigidity of 22 years to all crossdating procedures. Prior to dividing by the spline (detrending), growth increments and variance were much greater in the younger

Fig. 2. (*a*) Time series of growth increment widths of two otoliths, along with best-fit 22-year spline curves (broken lines). (*b*) Standardized increment-width indices in which measurements were divided by spline curve values. Both standardized otolith time series have a mean of 1 and homogenous variance. Note the synchrony in high-frequency growth patterns.



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portions of each otolith time series (Fig. 2*a*). Detrending with the spline resulted in a series of dimensionless increment-width indices with a mean value of 1 and relatively homogenous variance along the length of each time series (Fig. 2*b*).

For all 50 otoliths, the average correlation between each standardized otolith time series and the master chronology (interseries correlation) was 0.523. Seven otoliths had 50-year segments that did not significantly ($\alpha = 0.05$) correlate with the master chronology, suggesting potential problems. To further verify crossdating and help identify potential errors in specimens with low correlations, each 50-year segment in each otolith time series was lagged between plus or minus 1–10 years to determine whether shifting the series yielded a higher correlation with the master chronology. A higher correlation with a lagged year would indicate missed or added increments and that the otolith time series should be reexamined. To better illustrate this concept, we introduced an error into one of the otolith time series that had a correlation with the master chronology of 0.62. We simulated missing the narrow 1983 growth increment by combining its width with that of 1982. In so doing, all measurements were shifted 1 year from their true dates of formation. This error substantially reduced correlation to the master chronology ($r = -0.11$). More importantly, lagging the otolith time series by 1 year improved the correlation coefficient ($r = 0.42$). Such a difference between the position of zero lag and 1 year of lag clearly indicated that an increment was missed. Correlations for the seven potentially problematic otolith time series were not as dramatic as that in the above example, and in all otoliths, the average correlation was positive (ranging from 0.184 to 0.277). In five otoliths, lagging would increase correlations with the master chronology. However, these correlations are almost certainly spurious, since the lags were 5–8 years, and the correlations were only negligibly higher. Inspection of problematic otoliths indicated that, in some regions, boundaries between increments were relatively unclear, which likely accounted for the low correlation with the master chronology. Though COFECHA indicates relatively low correlations in these samples, we did not feel correlations were low enough to question the overall crossdating. Visual crossdating was strong, and some individuals may experience different environmental conditions or respond differently to environmental variability.

For perspective on the strength of the otolith results, interseries correlations of splitnose rockfish are comparable with those of late successional tree species growing in closed, temperate forests. Eastern hemlock (*Tsuga candensis*), sugar maple (*Acer saccharum*), and American beech (*Fagus grandifolia*) typically crossdate with interseries correlations between 0.5 and 0.6. Though interseries correlations are higher for species growing in environments in which climate is severely limiting, such as in deserts or tree-line forests, tree-ring data from closed, temperate forests significantly crossdate and correlate with climate variables (Cook and Cole 1991; Graumlich 1993; Tardif et al. 2001). The synchronous climate signal in eastern hemlock has even been used as proxy data for climate reconstructions (Cook and Cole 1991). Therefore, interseries correlations with the master chronology for splitnose rockfish are at levels considered fully acceptable by the dendrochronological community. This strongly suggests that climate induces a common signal in splitnose growth increments, and that increment widths can be used to validate age of splitnose rockfish with a high degree of precision.

The master chronology generated by averaging residual chronologies of all 50 otoliths correlated with the upwelling index at 33°N, 119°W ($r = 0.33$, $p = 0.01$), the upwelling index at 36°N, 122°W ($r = 0.40$, $p = 0.002$), the PDO ($r = -0.29$, $p = 0.007$), and the NOI ($r = 0.51$, $p = 0.0001$). These results indicate that environmental variability strongly affected rockfish growth. More importantly for this study, they provide strong evidence that the correct calendar years were assigned to the chronology and crossdated growth increments. If fluctuations in the otolith growth patterns correspond to records of ocean variability, otolith increments should be properly dated. The relationship between environmental variability and growth response is not lagged, and ring width primarily reflects conditions of the current year. For example, others have noted in *Sebastes* that the 1983 El Niño substantially reduced growth in the 1983 increment (Pearson 1996; Woodbury 1999; Love et al. 2002). As we crossdated from the margin of the otolith toward the focus, we found the exact number of rings expected to the 1983 signature year, given the date of fish capture. For example, the 1983 signature year occurred six increments prior to the partial ring at the margin for fish caught in the summer of 1989. If a lag occurred between the event and growth response, we would expect to reach the signature year in fewer growth increments than expected. However, this was not the case. Furthermore, no significant ($\alpha = 0.05$) correlations occur between the splitnose rockfish chronology and environmental variables lagged by 1 or even 2 years. Thus, we believe the chronology has been accurately placed in time, and can therefore be used for age validation.

Our goal to correlate the splitnose rockfish master chronology with variables of the physical environment was to validate crossdating accuracy. However, these correlations also begin to reveal how climate has induced natural markers in the otolith time series. As indicated by a positive correlation with the NOI and upwelling and a negative correlation with the PDO, cool ocean conditions and high levels of upwelling positively affected splitnose growth. El Niño events, warm phases of the PDO, and downwelling led to narrow growth increments in splitnose rockfish. These responses to climate are consistent with other studies that suggest warmer water temperatures and reduced upwelling can reduce rockfish growth (Boehlert et al. 1989). For example, El Niño appears to have had negative effects on widow and yellowtail rockfish growth, causing narrow growth increments to form during the severe 1983 event (Pearson 1996; Woodbury 1999). Low levels of upwelling, such as those that occur during El Niño events, reduce productivity in the ocean and consequently reduce food supply and growth for rockfishes. As evidence of reduced food availability, levels of visceral fat in yellowtail rockfish collected in 1983 were much lower than those in 1980 (Lenarz and Wyllie Echeverria 1986). Apparently, warm ocean conditions indicate periods of low ocean productivity and reduced growth in splitnose rockfish. Conversely, cooler ocean conditions appear to indicate periods of enhanced productivity, which

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leads to increased growth in splitnose rockfish. Such climatic variability has induced short-term variations in growth, which in turn allowed for such strong crossdating.

In conclusion, crossdating is unique in that it uses natural markers induced by climate to validate the calendar year of each growth increment in long-lived fishes. Other methods that provide annual resolution, such as mark and recapture, are not as effective at such long time scales, nor can they be applied to large number of samples. Also, existing techniques for estimating longevity of long-lived fishes, such as bomb radiocarbon or radioisotopic analysis, are only accurate to within a few years. Thus, crossdating results in a higher level of accuracy and precision than other available techniques. While crossdating currently is limited to individuals greater than 30 years of age, this could be extended to younger fish if the synchronous growth pattern is strong. Very short-lived fish would provide too few degrees of freedom by which to fit trend curves to the growth increment series and to correlate the series with a master chronology. Also, crossdating is limited to individuals that form clear boundaries between increments, which may eliminate some long-lived fish species until improved preparation techniques enhance visibility of otolith structure. Fish with an unknown date of death may be crossdated as well, though a skeleton-plotting technique may be more effective for these samples than the "list year" approach we used in this study (Yamaguchi 1991; Stokes and Smiley 1996).

Future research will be necessary to evaluate other fish species for successful crossdating. Most likely, crossdating will be appropriate for other long-lived rockfish species and additional marine and freshwater fishes, given the clarity of their otolith cross sections (Guyette and Rabeni 1995; Pereira et al. 1995; LeBreton and Beamish 2000). The spatial extent over which otolith are collected must also be explored. This present study includes rockfish from a geographic area spanning five degrees of latitude. Yet spatial variability across larger areas may induce region-specific otolith growth patterns, which would reduce interseries correlations and the power of crossdating. Also, crossdating may be confounded if fish are migrating. But once the appropriate spatial scale for successful crossdating is established, master chronologies could be developed for each species and location and made available on the Internet. Individual otoliths could then be crossdated against the existing master chronology such that a large number of otoliths would not be necessary to generate a master chronology every time crossdating is applied. This would greatly expedite crossdating and would allow for age validation from small samples of otoliths. Overall, we believe that crossdating provides accurate estimates of fish ages that should have wide application to a variety of long-lived species in marine and freshwater systems.

Acknowledgements

This research was supported by NOAA grant NA17FE2705 to the Cooperative Institute for Marine Resources Studies as part of the Fisheries and the Environment (FATE) program of NOAA fisheries. We also thank two anonymous reviewers for their helpful comments on the manuscript.

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Do bears facilitate transfer of salmon resources to aquatic macroinvertebrates?

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Abstract: In coastal areas of the Pacific Northwest bears (*Ursus* spp.) prey heavily on spawning Pacific salmon (*Oncorhynchus* spp.) and selectively kill energy-rich individuals that are the most recent arrivals on spawning grounds. Pacific salmon eventually die in spawning habitats anyway, albeit with considerably lower energetic content. We investigated whether foraging activities of bears facilitate growth of stream invertebrates by increasing the duration of salmon carcass availability and the nutritional value of carcasses for scavengers. Our survey in southwest Alaska showed that carcasses are highly colonized by caddisfly (Trichoptera) larvae. Caddisflies show a strong preference for bear-killed over senescent carcasses, which may be a result of extended temporal availability, improved accessibility of consumable tissue, and higher energetic content of bear-killed fish. Isotope analyses further indicate uptake of marine-derived nutrients in caddisflies during the salmon run, which, however, does not extend into subsequent generations. Thus, species with life histories linked to the annual marine derived nutrient pulse gain the biggest advantage from the salmon resource subsidy. A long-term survey in several creeks in this region showed that bear predation intensity varied greatly among creeks and years, therefore indirect effects of bear predation on aquatic scavengers are likely highly patchy in time and space.

Résumé : Dans les régions côtières pacifiques du nord-ouest américain, les ours (*Ursus* spp.) font une forte prédation sur les saumons du Pacifique (*Oncorhynchus* spp.) lors de la fraye et tuent de préférence les individus riches en énergie qui sont le plus récemment arrivés sur les sites de reproduction. Les saumons du Pacifique meurent sur les habitats de fraye de toute façon, bien que leur contenu énergique soit alors considérablement réduit. Nous avons examiné si les activités alimentaires des ours améliorent la croissance des invertébrés des cours d'eau en prolongeant la période de disponibilité des carcasses de saumons et en augmentant la valeur nutritive des carcasses pour les charognards. Notre inventaire dans le sud-ouest de l'Alaska montre que les carcasses sont fortement colonisées par les larves de phryganes (Trichoptera). Les trichoptères montrent une forte préférence pour les carcasses tuées par les ours par rapport aux carcasses sénescentes, ce qui peut s'expliquer par une disponibilité prolongée dans le temps, une accessibilité accrue de tissu consommable et un contenu énergétique supérieur des poissons tués par les ours. Les analyses d'isotopes indiquent de plus une accumulation de nutriments d'origine marine chez les trichoptères durant la montaison des saumons, qui, cependant, ne se prolonge pas dans les générations qui suivent. Ainsi, les espèces dont le cycle biologique est relié à l'apport ponctuel annuel de nutriments d'origine marine retirent un avantage maximal de la contribution des saumons aux ressources du milieu. Un inventaire de longue durée dans plusieurs ruisseaux de la région montre que l'intensité de la prédation par les ours varie considérablement d'un cours d'eau à un autre et d'une année à l'autre; les effets indirects de la prédation des ours sur les charognards aquatiques sont donc vraisemblablement très variables dans l'espace et le temps.

[Traduit par la Rédaction]

Introduction

Large carnivores have considerable influence on the structure and function of lower trophic levels by directly regulating the abundance of their prey, which in turn may indirectly affect other species with which carnivores do not interact directly. Well-known examples of such trophic cascades include predation by wolves on ungulates (moose) (Post et al. 2002), which in turn has strong effects on fir trees (McLaren and Peterson 1994), or predation by killer whales on sea ot-

Received 15 October 2004. Accepted 11 April 2005. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on 22 September 2005.
J18357

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 doi: 10.1139/F05-136 © 2005 NRC Canada

ters, which in turn strongly affects urchin densities and kelp production (Estes et al. 1998). Large carnivores can also cause physical and chemical changes to environments, thereby altering habitats used by other organisms and controlling the availability of resources for scavengers (Rose and Polis 1998; Wilmers et al. 2003). In coastal areas of the Pacific Northwest the foraging behavior of brown and black bears (*Ursus arctos* and *Ursus americanus*, respectively) on spawning anadromous salmon (*Oncorhynchus* spp.) modifies the structure of riparian areas (Frame 1974; Gende et al. 2002). Salmon populations and subsequent bear predation are especially remarkable in coastal Alaska and British Columbia, where bears kill large quantities of salmon when they are available (Hilderbrand et al. 1996; Reimchen 2000), and carry partially consumed carcasses into riparian forests (Reimchen 1994; Gende et al. 2001, 2004*b*). In some regions, rates of bear predation on salmon are so high that a large fraction of the total salmon run are killed after only a few days in spawning creeks (Quinn and Buck 2000). Bears show a strong preference for energy-rich fish (those that had not spawned) and target energy-rich body parts including the brain and gonads (Gende et al. 2001, 2004*a*; Quinn et al. 2003). As a consequence, bear-killed salmon carcasses serve as an important resource for aquatic and terrestrial scavengers, including birds (Schindler et al. 2003).

The nutrient and energy subsidies to coastal ecosystems represented by spawning migrations of anadromous salmon have gained considerable attention from the aquatic sciences during the last decade (Cederholm et al. 1999; Gresh et al. 2000; Naiman et al. 2002). The salmon that return annually to Pacific Northwest freshwater systems are an important source of nutrients for aquatic biota (Bilby et al. 1996; Naiman et al. 2002; Ito 2003) and riparian plants and consumers (Kyle et al. 1997; Helfield and Naiman 2001; Ben-David et al. 2004). Studies using stable-isotope tracers such as nitrogen (^{15}N) and carbon (^{13}C) demonstrate that salmon nutrients are incorporated into stream biota and riparian plants at various trophic levels (Bilby et al. 1996), and that marine-derived N can represent 30%–75% of the N in fishes and aquatic invertebrates (Naiman et al. 2002). This fertilization process provides high-energy food for resident fishes, juvenile salmon, and invertebrates, and can increase aquatic- and riparian-ecosystem productivity (Wipfli et al. 1998, 1999) through either direct consumption of salmon body tissue and gametes (Chaloner et al. 2002; Minakawa et al. 2002), increases in prey quality and abundance, or leaching of nutrients from decaying salmon carcasses (Bilby et al. 1996).

Upon entry into fresh waters, salmon cease feeding, which can be several months before spawning commences. During freshwater migration and spawning, salmon lose 80%–90% of their lipid and about 40%–50% of their total energy (Hendry and Berg 1999; Gende et al. 2004*a*). Salmon live generally for less than 3 weeks on the spawning grounds (Qu'an et al. 2001*a*), but this varies among populations. The physiological deterioration associated with prolonged migration and spawning leads eventually to death. Therefore, the nutritional value of salmon carcasses depends strongly on the timing of death relative to the timing of entry into fresh water (Gende et al. 2002). Salmon killed immediately after entering spawning creeks (either by bears or through being stranded naturally because the water level is low) have a higher nutritional value than those senescing after many days in the creek (Gende et al. 2004*a*).

The decomposition of salmon carcasses can take several months to complete, because although the rate of decomposition is initially high, it declines over time (Chaloner et al. 2002). As with terrestrial carrion (Hanski 1987), scavengers are important agents in the decomposition of salmon carcasses (Britton and Morton 1994; Chaloner et al. 2002; Meehan et al. 2005). Avian and other vertebrate scavengers often remove chunks of salmon tissue and may carry some carcasses into riparian areas. Carcasses remaining in creeks are colonized by a range of terrestrial and aquatic invertebrates (Kline et al. 1997; Chaloner et al. 2002; Nakajima and Ito 2003) that scavenge salmon tissue. Caddisfly (Trichoptera) larvae are often the numerically dominant insect group associated with salmon carcasses (Minakawa et al. 2002), although many other insect taxa have been identified on salmon carcasses. Invertebrates likely facilitate the transfer of nutrients and energy from salmon carcasses to other components of the food web and therefore the transfer of marine-derived nutrients (MDN) to higher trophic levels (Bilby et al. 1998).

The objective of this study was to investigate whether brown bear predation on sockeye salmon (*Oncorhynchus nerka*) facilitates the growth of aquatic scavengers. We hypothesized that bear-killed salmon carcasses represent high-quality food resources for aquatic scavengers, which, in turn, opportunistically and preferentially feed on these carcasses as a means to maximize their own growth and development rates. Specifically, we surveyed the impact of bear predation on sockeye salmon over the season in Hidden Lake Creek, southwest Alaska, and investigated the colonization of salmon carcasses by caddisfly larvae. We monitored the energetic and nutritional response of caddisfly larvae to the presence of sockeye salmon carcasses that accumulated over the course of seasonal spawning activities of this population. In addition, we used a long-term data set of carcass counts in several creeks in this region to estimate the temporal and spatial variation of bear predation on migrating sockeye salmon, which allowed us to extend the survey from Hidden Lake Creek to a broader spatial and temporal scale. Our results highlight the importance of terrestrial predators for facilitating the propagation of MDN and energy into freshwater food webs.

Study site and methods

This study was conducted in the Wood River system located in the Wood–Tikchik State Park, southwest Alaska (59–60°N, 158–159°W) (for a map see Quinn et al. 2001*b*). Streams used as spawning grounds for salmon range in width from 1.7 to 15.7 m and are described in detail in Marriott (1964). Sockeye salmon dominate the spawning runs of this region between late July and mid-October, with peak runs in late July to early September. Live and dead salmon were surveyed in 25 creeks throughout the Wood River system during peak spawning (August) by walking upstream from the mouth of the creek until a barrier to migration or a region with unsuitable habitat for salmon was

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reached. Carcasses in the stream channel and riparian zone were categorized by mode of death (i.e., senescent or bear-killed). Sampling frequency varied among creeks and resulted in 4–17 years of data for each creek. Based on these annual surveys the proportion of bear-killed sockeye salmon in each of these creeks was calculated according to Quinn et al. (2001*b*, 2003) as the average of the following ratios: bear kills/all dead and bear kills/(live + all dead). The average of these two predation levels takes into account the seasonal variation of bear-killed sockeye salmon in relation to sockeye salmon density and is an accurate estimate throughout most of the salmon run (Quinn et al. 2003).

In 2003, a 400-m reach of Hidden Lake Creek was surveyed over the course of the salmon run to monitor changes in the densities of salmon carcasses available to aquatic scavengers. The section of the creek was, on average, 0.5 m deep and 3 m wide. Sockeye salmon carcasses were counted and categorized by their mode of death (stranded, senescent, bear-killed) once a week from 27 July to 25 August. Muscle tissue from the middorsal area of fresh carcasses (bear-killed or stranded; $N = 27$) and senescent carcasses ($N = 10$) was taken at the end of July and middle of August, frozen, and later freeze-dried for 18 h to determine energy density and stable-isotope characteristics. In terms of composition, dorsal muscle is a good representative of the entire soma (Hendry and Berg 1999). Each sample was ground and the calorimetric value was determined using a 1425 Semimicro Bomb Calorimeter (Paar Instrument Company, Moline, Illinois). According to Hendry and Berg (1999) a water content of 71% and 83% was assumed for fresh and senescent carcasses, respectively. In addition, isotope ratios of $\delta^{15}N$ and $\delta^{13}C$ were measured for each of the carcasses (for descriptions see below).

To estimate the biovolume of bear-killed salmon carcasses and the proportion of bear-killed carcass remnants in the creek, we measured the weight and snout length of fresh bear-killed carcasses (36 females and 28 males) and of live ripe (20 males and 20 females) sockeye salmon. From the snout length – weight regression for live sockeye salmon (females: $r^2 = 0.79$, $P < 0.001$; males: $r^2 = 0.90$, $P < 0.001$), the original weight of the carcasses was estimated and the average proportion of biomass consumed per carcass calculated.

In the same section of Hidden Lake Creek, the abundance of caddisfly (Trichoptera, Limnophilidae, mainly *Ecclisomyia* sp.) larvae (Wiggins 1977) on carcasses was determined on each survey date by rotating the carcasses on the stream bed in shallow water or gently lifting them onto a submerged tray in deeper water to prevent loss of caddisfly larvae from them. Our visual inspection suggested that this method was effective on both types of carcasses and did not bias our estimates of higher densities of caddisflies on bear-killed carcasses. Both external and exposed internal body parts were carefully observed for caddisflies, and carcasses were assigned to the fresh (bear-killed, stranded) or senescent category. Carcasses are distinguishable as follows: bear-killed carcasses have holes from canine teeth and a varying amount of tissue has been removed; stranded carcasses are intact and fresh, with negligible wear on the fins; senescent carcasses are emaciated and have dried and tattered fins and tails as a result of nest-digging, and fungus on the body. Selection by caddisfly larvae of bear-killed carcasses was quantified by Chesson's selectivity coefficient, α (Chesson 1983), which is a measure of preference for bear-killed over senescent carcasses. This index was calculated as

$$(1) \qquad \alpha = \frac{r_i/n_i}{\sum r_j/n_j}$$

where r_i is the proportion of caddisfly larvae on bear-killed carcasses, n_i is the proportion of bear-killed carcasses in the creek, and r_j and n_j are the proportion of caddisfly larvae on bear-killed and senescent carcasses, respectively. Stranded carcasses were excluded from the analysis because of their rare occurrence. In our study, α values of 0.5 represent random colonization (1/number of types of food available) and α values greater or less than 0.5 represent selection and avoidance of bear-killed carcasses, respectively.

In addition to those that were collected from the carcasses, caddisfly larvae were also collected from the creek bed using a Surber sampler (30.5 cm × 30.5 cm) from 4 July to 25 August. Caddisflies were preserved in 70% ethanol for growth, energy-content, and isotope analyses. Caddisfly larvae from two nearby non-salmon creeks (N-4 and Upper Pick) were also collected for isotope analyses between 21 July and 4 September.

In the laboratory, caddisflies were removed from their cases, measured to the nearest 1 mm of length, dried for 18 h in a freeze-dryer, and weighed to the nearest 0.001 mg. Growth rates for caddisflies were measured as the difference in dry weight (DW) over time ($N = 26$–125 individuals per date). On each sampling date, the energetic densities of individual caddisflies sampled from the creek and from carcasses were measured by bomb calorimetry ($N = 5$ individuals per date; on 4 July only three individuals were available). Further, we used $\delta^{13}C$ and $\delta^{15}N$ ratios to measure the input of salmon-derived C and N in caddisflies ($N = 5$–6 individuals per date) collected from the creek bed and carcasses. Dried caddisflies were ground and enclosed in tin capsules. Isotope analysis was conducted at the Stable Isotope Facility, University of California Davis. Stable-isotope ratios are expressed as $\delta^{13}C$ and $\delta^{15}N$ values (Peterson and Fry 1987). These values represent the level of enrichment or depletion of the heavier isotope of N or C relative to a standard. Values were calculated as follows:

$$(2) \qquad \delta^{13}C \text{ or } \delta^{15}N = \frac{R_{sample} - R_{standard}}{R_{standard}} \times 1000$$

where R is the ratio of heavy isotope to light isotope. Standards are air for N and Pee Dee Belemnite for C. Higher $\delta^{15}N$ or $\delta^{13}C$ values indicate higher proportions of the heavier isotope in the sample. Because salmon are enriched in ^{13}C and ^{15}N relative to freshwater and terrestrial systems, elevated ^{13}C:^{12}C or ^{15}N:^{14}N ratios in freshwater systems are generally indicative of marine (salmon) enrichment (Schoeninger et al. 1983; Owens 1987).

The observed isotope values in the caddisflies were converted to percentages of marine-derived N using a two-source mixing model (Bilby et al. 1996). This model calculates the percentage of marine-derived N as

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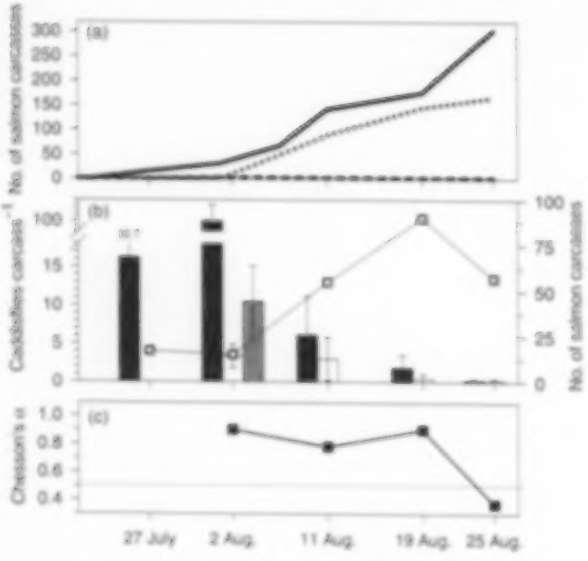
Fig. 1. Sockeye salmon (*Oncorhynchus nerka*) carcasses and caddisfly (Trichoptera) larvae in Hidden Lake Creek over the summer season in 2003. (*a*) Cumulative abundance of salmon carcasses in the creek characterized by their mode of death (bear killed (—), senescent (···), or stranded (- · -)). (*b*) Number of caddisfly larvae per sockeye salmon carcass characterized by the mode of death of the salmon (bear-killed (solid bars), senescent (open bars), or stranded (shaded bars)) and the number of salmon carcasses counted (□). Values are shown as the mean ± standard error. (*c*) Mean Chesson's selectivity coefficients, α, for bear-killed carcasses by caddisfly larvae (■). The dotted line indicates random feeding (α = 0.5). Values >0.5 represent positive selection of bear-killed carcasses; values <0.5 represent avoidance.



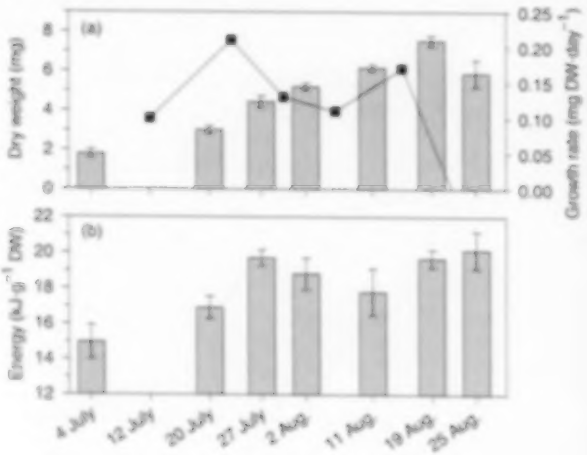
$$\%\text{MD-N} = \frac{(\delta^{15}N_{caddis} - 3.4) - (\delta^{15}N_{base} - 3.4)}{\delta^{15}N_{salmon} - (\delta^{15}N_{base} - 3.4)} \times 100 \quad (3)$$

where %MD-N is the percentage of marine-derived N; $\delta^{15}N_{caddis}$ is the observed isotope value of the sample; $\delta^{15}N_{salmon}$ is the marine end member (i.e., isotope values representing 100% marine-derived N from average salmon isotope values); $\delta^{15}N_{base}$ is the caddisfly baseline (i.e., isotope values representing 0% marine-derived N; this is the average value for caddisfly larvae sampled from two nearby non-salmon creeks); and 3.4 is the assumed increase in $\delta^{15}N$ per trophic level (DeNiro and Epstein 1981; Vander Zanden and Rasmussen 1999).

Results

In Hidden Lake Creek, sockeye salmon were first observed on 27 July (Fig. 1*a*). Almost all of the early carcasses were produced via bear predation and a few carcasses were stranded. No carcasses of senescent salmon were observed until 2 August. From early August onwards, the number of bear-killed and senescent carcasses increased, with bear-killing being always the dominant mode of death. On average, bear-killed carcasses retained 80% of their original body mass and the biomass of bear-killed carcasses in the creek ranged from 4.8 to 39.2 g DW·m^{-2} during the salmon run.

Fig. 2. Condition of caddisfly larvae in Hidden Lake Creek over the summer season in 2003. (*a*) Seasonal dry weight (DW; bars (mean ± standard error)) and instantaneous growth rate (■) for caddisfly larvae. The dotted line denotes a rapid decrease in growth rate, which was probably driven by pupation of the larvae. (*b*) Seasonal energy content of caddisfly larvae (mean ± standard error).



In Hidden Lake Creek, sockeye salmon carcasses were heavily colonized by caddisfly larvae, which reached the highest densities in early August (average density 68·carcass^{-1}, maximum density 300·carcass^{-1}). Caddisflies were found on the carcass surface, buried within carcass tissue, in gill, mouth, and brain cavities, and in cavities created by bear bites. Based on the survey in Hidden Lake Creek, we found more individual caddisflies on body fragments of carcasses (i.e., bear-killed carcasses) (average ± standard error (SE) = 24.6 ± 17.1) than on senescent (1.7 ± 1.5) or stranded carcasses (2.1 ± 1.2) (Fig. 1*b*). Chesson's selectivity coefficients (bear-killed : senescent carcasses) calculated for caddisfly larvae indicated a strong preference for bear-killed over senescent carcasses from July until mid-August and random feeding on carcasses in late August (Fig. 1*c*).

The energetic content of bear-killed carcasses was significantly higher than that of senescent carcasses (analysis of variance (ANOVA), $F_{[1,35]} = 1962.1$, $P < 0.001$) with a value of 6.55 ± 0.04 (average ± SE) kJ·g^{-1} wet weight in bear-killed ones and 3.68 ± 0.03 kJ·g^{-1} wet weight in senescent ones. In contrast, the stable-isotope ratios $\delta^{15}N$:$\delta^{14}N$ and $\delta^{13}C$:$\delta^{12}C$ did not differ between bear-killed and senescent carcasses (Fig. 3).

The average DW of individual caddisfly larvae increased progressively over the season from 1.8 mg in early July to a peak value of 7.5 mg in mid-August (Fig. 2*a*). Average caddisfly biovolume ranged from 2.3 to 6.3 mg DW·m^{-2} in the creek and from 0.3 to 5.3 mg DW·m^{-2} on the carcasses. The biomass of caddisflies on bear-killed carcasses represented only a small proportion of that of the carcass (always less than 0.14%). By the end of July, the caddisfly growth rate peaked and was relatively high throughout the rest of

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Fig. 3. The $\delta^{13}C$ and $\delta^{15}N$ ratios (mean ± standard error) of caddisfly larvae before and during the salmon run and in muscle tissue of bear-killed (fresh) and senescent sockeye salmon in Hidden Lake Creek over the summer season in 2003. In addition, isotope values from two nearby non-salmon creeks (Upper Pick and N-4) are shown before the salmon entered the system on 27 July, and in N-4 during the whole period of the salmon run. Isotope values for caddisflies in Hidden Lake Creek are shown before and over the course of the salmon run (solid symbols represent caddisflies sampled from the creek bed and open symbols represent caddisflies sampled directly from carcasses). Numbers in parentheses are days of the month.

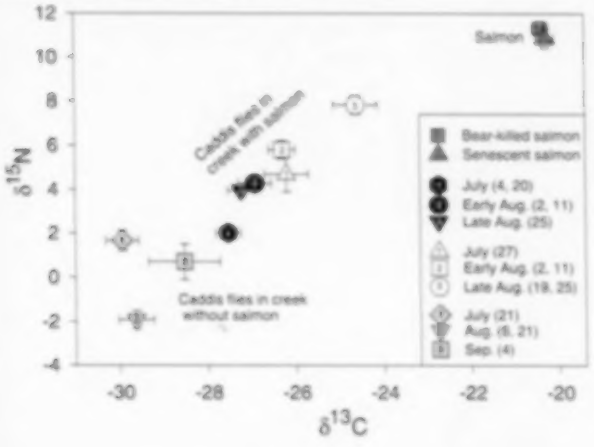
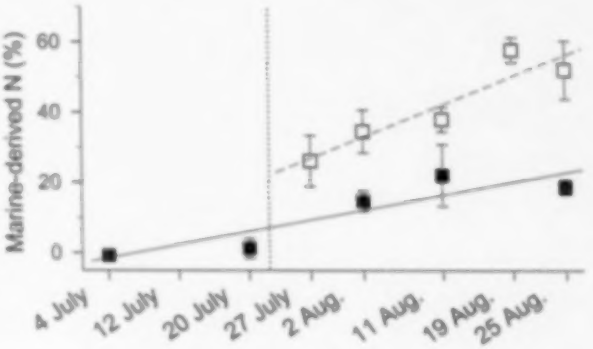


Fig. 4. Percentages of marine-derived N in caddisfly larvae sampled from the creek bed (■) and from salmon carcasses (□) over the summer season in 2003 in Hidden Lake Creek, showing linear regression lines. The dotted line indicates the date when salmon were first observed in the creek. Values are given as the mean ± standard error.



the summer (Fig. 2*a*). The energy content increased from 14.96 ± 0.97 (average ± SE) to 20.13 ±1.04 kJ·g^{-1} DW during the summer (Fig. 2*b*). In addition, the $\delta^{15}N$ and $\delta^{13}C$ signal in caddisflies from the creek increased progressively over the summer after salmon entered the creek, but decreased again in late August (Fig. 3). Stable-isotope values were always higher in caddisflies taken from carcasses than in those collected from the creek bed during the salmon run (ANOVA, $\delta^{15}N$: $F_{[1,30]} = 22.37$, $P < 0.001$; $\delta^{13}C$: $F_{[1,30]} = 9.71$, $P < 0.005$; this includes dates when samples were taken simultaneously from the creek bed and carcasses). The stable-isotope ratio for caddisfly larvae in Hidden Lake Creek approached that measured in salmon carcasses over the month of August, but did not increase for caddisfly larvae in a nearby non-salmon creek (Fig. 4). Consequently, the percentage of marine-derived N in caddisflies increased progressively over the season, with a maximum of about 58% in individuals sampled from the carcasses and 22% in individuals sampled from the creek (Fig. 4). The energy content and DW of caddisflies did not differ between individuals sampled simultaneously from the carcasses and creek bed (ANOVA, energy: $F_{[1,34]} = 0.91$, $P = 0.35$; DW: $F_{[1,264]} = 0.43$, $P = 0.51$; data not shown).

A long-term survey of 25 creeks in the Wood River system indicated that the impact of bear predation as the primary mode of death of migrating sockeye salmon varied considerably in both space and time. The average proportion of bear-killed salmon carcasses ranged from 5% to 82% among creeks between 1970 and 2003 (Fig. 5), and also varied greatly among years within a single creek, especially in creeks with moderate bear predation. However, some creeks always had relatively low or high rates of bear predation across the entire survey period.

Discussion

The foraging behavior of brown and black bears can have a strong impact on the mode of death of migrating salmon (Quinn and Buck 2000; Gende et al. 2001). Our survey supports the results of Quinn et al. (2003) and Gende et al. (2004*a*), showing that nearly all salmon arriving early in the spawning run were killed by bears, and that the number of senescent carcasses increased later in the season with increasing salmon density. In Hidden Lake Creek bear-killed sockeye salmon carcasses were available to aquatic scavengers about a week before the carcasses of senescent individuals were observed. Throughout the salmon run the proportion of bear-killed carcasses was always slightly higher than that of senescent ones. A similar pattern with a relatively high proportion of bear-killed carcasses was reflected, on average, over 16 years (~45% bear-killed carcasses) in Hidden Lake Creek. In this creek sockeye salmon carcasses were colonized by caddisfly larvae (mainly *Ecclisomyia* sp.) especially between late July and early August, with a maximum density of 300 individuals per carcass. The association of caddisflies and other macroinvertebrates with salmon carcasses has been also shown in other studies throughout the Pacific Northwest (Chaloner et al. 2002; Minakawa et al. 2002).

Our study shows that caddisfly larvae selectively prefer the carcasses of bear-killed salmon, especially until mid-August, while later in the course of the salmon run, bear-killed and senescent carcasses were colonized equally. Two mechanisms may drive this preference for bear-killed carcasses. First, since bears often target specific body parts (Gende et al. 2001) and therefore open up salmon carcasses, improved accessibility of consumable tissue (i.e., muscle, brain, and viscera) may explain the dominant colonization of

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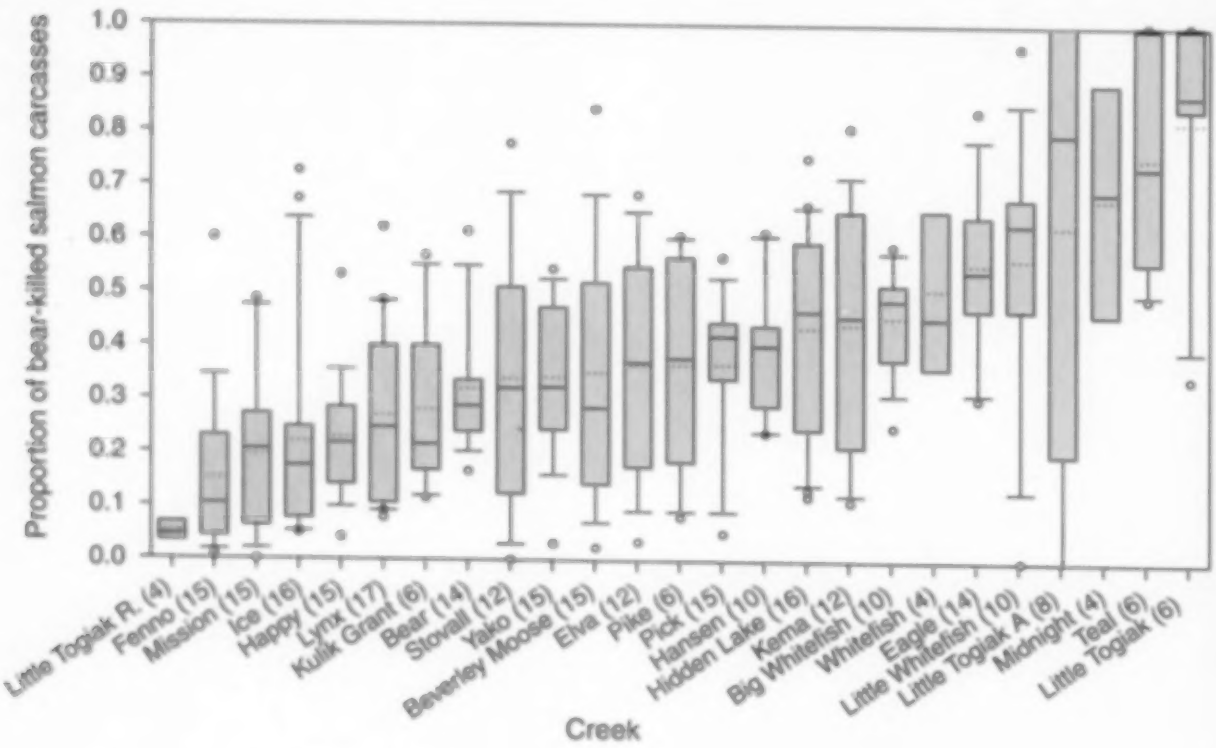
Fig. 5. Box plots showing the proportions of bear-killed sockeye salmon carcasses in 25 creeks in the Wood River system in southwest Alaska. Plots indicate the variation of counts in August across time series between 4 and 17 years (the number of years is given in parentheses). Creeks are ordered from low to high bear predation rates. Each box represents the 25th and 75th percentiles; the dotted line indicates the mean, the solid line indicates the median, the vertical bars indicate the range excluding outliers, and the circles outside the bars indicate outliers.



bear-killed remains. Gaining access to muscle tissue of stranded or senescent carcasses is more difficult because caddisflies have to first penetrate the tough salmon skin. Second, bear-killed carcasses may also be a resource that provides a higher energetic and nutritional level than senescent carcasses (Hendry and Berg 1999). Therefore, in the Pacific Northwest, bears, but also other mammals and birds that kill and consume salmon (Jauquet et al. 2003), indirectly facilitate the growth of scavengers by altering the temporal availability, accessibility, and quality of an important food resource. In areas where bears are absent, the foraging activities of other salmon predators like wolves, mink, eagles, and gulls are expected to have a similar function to those of bears and may also indirectly affect the growth of scavengers.

In sites with low salmon densities and high bear-activity levels it is likely, however, that bears reduce salmon resource subsidies available to macroinvertebrates, especially if bears consume high proportions of salmon and carry them into the riparian zone (Reimchen 1994; Meehan et al. 2005). However, in the Wood River system salmon runs are usually high (Hilborn et al. 2003) and the proportion of salmon removed by predators from the creek bed is usually low (Meehan et al. 2005), except for very small creeks, where bears may remove up to 50% of carcasses from the creek bed. In addition, when salmon densities are high, bears usually consume only a small fraction (20%, on average, as shown in our study) and thus provide a resource subsidy to scavengers by provisioning them with the remains of their kills.

The incorporation of MDN in caddisfly larvae was indicated by the immediate increase in $\delta^{15}N$ coincident with the arrival of sockeye salmon in Hidden Lake Creek and by the gradual enrichment of $\delta^{15}N$ and $\delta^{13}C$ in caddisflies over the course of the salmon run, approaching levels measured in salmon carcasses. In contrast, caddisfly larvae from a non-salmon creek showed no enrichment in $\delta^{15}N$ during the course of the salmon run. The similarity in $\delta^{15}N$ values between caddisflies from two nearby non-salmon creeks and from Hidden Lake Creek before salmon arrived suggests that the background uptake of marine-derived N is negligible and that MDN likely do not extend into subsequent generations of caddisflies. This indicates that MDN are not retained long enough in the system to affect growth early in the season; this was also proposed by Lessard et al. (2003). The difference in caddisfly $\delta^{13}C$ value between the non-salmon creeks and Hidden Lake Creek may be driven by geomorphological and hydrologic factors, which can strongly influence the isotopic source of C available to consumers (Finlay et al. 2003), and the differences in $\delta^{15}N$ values between the creeks may reflect differences in feeding patterns. Our study indi-

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cates that the increase of MDN in caddisfly larvae is temporary in this system and is strongly associated with the presence of salmon. The temporal increase in consumers' productivity during the course of the salmon run has also been shown in other invertebrates and fish (Wipfli et al. 1998; Zhang et al. 2003). Thus, species with life histories linked to the annual MDN pulse may gain the biggest advantage from the salmon resource subsidy. Invertebrates with autumnal reproduction have access to a particularly rich food resource to prepare for pupation and reproduction, and the reproductive success of these consumers may be strongly affected or even be limited by the availability of salmon-derived resources.

The enrichment of marine-derived N and C in caddisfly larvae sampled from the creek at large and from carcasses indicates that some MDN are incorporated indirectly via detritus or direct uptake by scavenging on carcasses. Incorporation of marine-derived N in caddisflies ranged up to 58% and the enrichment of N was always higher in caddisflies sampled directly from carcasses than in individuals sampled from the creek bed, although the energy content did not vary between these two classes of caddisflies. In addition, leaf litter fall may also contribute to the increase in caddisflies' isotope signature (Helfield and Naiman 2001). The progressive increase in energy content and isotope values shows that salmon subsidies not only increase resource availability, but also improve food quality. The C:N ratio of caddisflies decreased with increasing salmon density and therefore also the nutritional value of caddisfly larvae and their value as prey increased. Therefore, salmon not only represent a highly nutritional prey source, but also increase the nutritional value of other components of the ecosystem (e.g., in prey species for resident fishes and juvenile salmon). Because foraging activities by bears increase the availability of the highest quality salmon carcasses, bears indirectly facilitate consumption of salmon by, and increase growth rates of, caddisflies and possibly other aquatic scavengers.

The life cycle of the caddisfly *Ecclisomyia* sp. in Hidden Lake Creek appears to be timed to coincide with the seasonal availability of salmon, with most larval growth occurring in midsummer at the time when salmon start to arrive, are killed by bears, and decompose. The relatively strong increase of the growth rate of caddisflies in this creek after the arrival of salmon suggests that caddisfly growth is strongly influenced by the availability of salmon-derived energy. An increase in growth after the arrival of salmon has also been shown for resident fishes and juvenile salmon in southeast Washington (Bilby et al. 1998). In addition, a laboratory study showed that *Ecclisomyia* sp. grew faster when reared on salmon carcasses than when reared on alder leaves (Minakawa et al. 2002). Apparently these detritivores have the appropriate feeding morphology and behavior required to switch from a primarily detritus-based diet to salmon tissue (Anderson 1973). Since animal material provides high-quality food with proportionally more digestible protein and essential nutrients (Anderson and Cummings 1979), salmon carcasses may be an essential energy source for the completion of larval stages and pupation of invertebrate larvae (Wiggins 1977). The decline in DW and isotope signal in late August may be driven by pupation and emergence of individuals growing the fastest, while those that remain may have fed less on salmon and achieved a slower growth rate and lower $\delta^{15}N$ and $\delta^{13}C$ values.

Bears are an important agent in the transport of nutrients from salmon carcasses (Quinn et al. 2003), and consequently affect growth of macroinvertebrates on a large scale. As was shown by Quinn et al. (2003) and by our survey of several creeks over a longer time period in southwest Alaska, the proportion of salmon killed by bears shows both spatial and temporal variation, suggesting that the provision of marine-derived subsidies for scavengers is not homogeneous and can vary greatly among years and sites. The variation in the proportion of salmon killed among creeks depends strongly on the physical characteristics of the creeks, which influence the availability of salmon to bears, the overall salmon density in the creek, and the density of bears using the creek (Gende et al. 2001, 2004*a*; Quinn et al. 2003). In general, bear predation rates are highest in the narrowest and shallowest spawning streams and lowest in more complex larger streams (Gende et al. 2004*a*). Therefore, the amount of resource subsidies to macroinvertebrates provided by bear-killed carcasses varies greatly on temporal and spatial scales associated with creek morphology, salmon densities, and bear activities.

In the Pacific Northwest, bears serve as important vectors of salmon-derived nutrients into riparian ecosystems (Reimchen 1994; Hilderbrand et al. 1999; Helfield and Naiman 2006). In addition, our study suggests that bears are important agents affecting the availability, accessibility, and nutritional quality of resources, like salmon, for scavengers, including caddisfly larvae, and thereby indirectly facilitate their growth and increase their nutritional value as prey. These results further emphasize the importance of large carnivores as keystone species in coastal ecosystems.

Acknowledgments

We thank all the people who helped in the field, especially Curtis Brock and Gordon Holtgrieve, and the numerous assistants who have performed the spawning-ground surveys for the University of Washington Alaska Salmon Program. Don Rogers initiated the long-term studies of bear predation on salmon in the Wood River System. M.W. is supported by the Austrian Science Fund (Fonds zur Foerderung der wissenscaftlichen Forschung, FWF). Financial support was provided by the National Science Foundation and the University of Washington.

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Studying the spatiotemporal variation of the littoral fish community in a large prealpine lake, using self-organizing mapping

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Abstract: One of the most fundamental feature of freshwater systems is the spatiotemporal structure of their communities. In the present study, we used an artificial neural network model, i.e., self-organizing mapping, together with a likelihood ratio χ^2 statistic for proportions to investigate the influence of each factor of a complex sampling scheme (i.e., site, year, month, and time of day) on the littoral fish community of Lake Constance (south Germany). Based on self-organizing mapping, four clusters of samples were defined characterized by distinct fish communities. The samples gathered in clusters 1 and 2 were significantly related to the factors month and time of the day, while those in cluster 3 were related to the factors month and site and those in cluster 4 to each of the four factors. The results are discussed with regard to the temporal patterns of species succession in lakes and their similarities with the spatial patterns observable in streams, the importance of plasticity with regard to the fish nycthemeral preferences, the partitioning of habitat at a large spatial scale and its importance for the coexistence of species, and the effects of the reoligotrophication process in lakes.

Résumé : L'une des caractéristiques fondamentales des écosystèmes d'eau douce est la structure spatio-temporelle de leurs communautés. Dans cette étude, nous avons utilisé un réseau de neurones artificiels du type carte auto-organisatrice, associé à des tests sur les proportions, afin de mettre en évidence l'influence de facteurs d'un plan d'échantillonnage complexe (site, année, mois et période de la journée) sur la communauté de poissons de la zone littorale du lac de Constance (sud de l'Allemagne). Les résultats de la carte auto-organisatrice nous ont permis de définir quatre groupes d'échantillons, caractérisés par des peuplements distincts de poissons. Les échantillons des groupes 1 et 2 étaient significativement liés aux facteurs mois et période de la journée, ceux du groupe 3 aux facteurs mois et site d'échantillonnage et ceux du groupe 4 à chacun des quatre facteurs. Les résultats ont été discutés en considérant les patrons de succession temporels observables en lac et leurs similarités avec les patrons spatiaux observables en cours d'eau, l'importance de la plasticité dans l'occupation du nycthémère, le partage de l'habitat à large échelle et son rôle dans la coexistence des espèces et les effets du processus de re-oligotrophisation en lac.

Introduction

Ecosystems are structured in both space and time. Consequently, one of the most fundamental questions when studying an ecosystem and its component organisms is the strategy of its occupation with respect to space and time, and sampling plans built according to both spatial and temporal factors are often used by ecologists to address this issue.

The littoral areas of lakes are among the most diverse and complex habitats in aquatic ecosystems. Habitat and food resources here are normally more diverse than in other lake compartments (Pierce et al. 1994), resulting in a higher abundance and diversity of fish fauna (Keast 1985). Moreover, most freshwater fish, including those regarded as typically pelagic, often use the littoral zone for a short period of the year, either for spawning (Gafny et al. 1992) or during larval and juvenile development (Allen 1982; Werner et al. 1983). Nevertheless, studies examining the spatial and temporal variation in littoral zone fish communities are remarkably sparse (Hinch and Collins 1993; Pierce et al. 1994; Rundle and Jackson 1996).

Lake Constance (Germany) is the second largest prealpine lake in Europe. The fish community of Lake Constance comprises 33 species from 11 families (Eckmann and Rösch 1998) of which more than 95% are either obligate or facultative users of littoral resources. Previous works of Fischer

Received 7 January 2005. Accepted 23 March 2005. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on 22 September 2005.
J18492

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 doi: 10.1139/F05-097 © 2005 NRC Canada

and Eckmann (1997*a*, 1997*b*) have shown that abundances of littoral fish species in Lake Constance exhibit very high spatial and temporal variation at seasonal and diel scales. Based on these results, a routine sampling program was started in 1997 to assess the influence of temporal and spatial factors, i.e., year, month, site, and time of day, on the littoral fish community over longer time periods.

The complex nature of community data has been a catalyst for the rapid development and transfer of multivariate methods in ecology (Rundle and Jackson 1996). Ordination techniques, designed to summarize and simplify large data sets, can help to elucidate factors contributing to the structure of the community under consideration (Ludwig and Reynolds 1988). While multivariate approaches may offer an appropriate means for examining the spatial and temporal variation in an ecosystem (Resh and Rosenberg 1989), examples of these kinds of approaches are rare (Rundle and Jackson 1996).

Artificial neural networks provide a powerful, flexible learning technique for uncovering patterns in ecological data (e.g., Lek and Guégan 2000; Reyjol et al. 2001*a*; Olden and Jackson 2002). Among artificial neural networks, self-organizing mapping (SOM) (Kohonen 1982) is an unsupervised model that acts as a nonlinear clustering technique that has been previously used to characterize distribution patterns of ecological systems (Chon et al. 1996; Giraudel and Lek 2001; Park et al. 2003). It shares with conventional ordination methods the basic goal of displaying a multidimensional data set in a lower, usually two-dimensional, space. With SOM, the data set is projected in a nonlinear way onto a rectangular grid laid out on a hexagonal lattice, i.e., the Kohonen map.

The objective of this study was twofold. In terms of introducing new methodology, we have used SOM to cluster the data to test specific hypotheses concerning spatial and temporal variation. This study is similar in spirit to Rundle and Jackson (1996), who used an ordination technique (principal components analysis (PCA)) for the same purpose.

From an ecological point of view, we wanted to investigate the spatiotemporal variation of the littoral fish community of Lake Constance. Few studies have investigated this general aspect of lake functioning (Hinch and Collins 1993; Pierce et al. 1994; Rundle and Jackson 1996), despite the fact that littoral zones usually provide food, shelter against large predators, and favorable thermal conditions for a variety of species during most of the year (Hinch and Collins 1993; Pierce et al. 1994; Fischer and Eckmann 1997*b*).

Lake Constance has been characterized as having a high level of spatiotemporal variation (Fischer and Eckmann 1997*a*, 1997*b*), and as such, it represents a relevant study system to bring new general insights concerning the spatiotemporal variation in littoral fish communities in lakes. This study is the first to gather information on such a large number of spatiotemporal factors of variation for littoral fish communities.

Materials and methods

Study area, sampling sites, and procedure

Lake Constance is the second largest, warm-monomictic lake north of the Alps mountain range. It is situated at the boundary of Austria, Germany, and Switzerland (47°N, 09°E) at an altitude of 395 m. Rivers entering the lake drain an area of 11 500 km^2 with two thirds of the water input originating from the River Rhine. The lake is morphologically divided in two basins (Kiefer 1972), the deep oligomesotrophic "Upper Lake" and the mesotrophic shallow "Lower Lake", which are connected by the River Rhine (Fig. 1). The Upper Lake constitutes the major part of the lake, with a surface area of 475 km^2, a mean depth of 95 m (maximum 254 m), and a shoreline length of 186 km. Its littoral zone (sensu Wetzel 1983) extends to a water depth of about 5 m during high-water conditions in summer and covers about 10% of the entire lake area.

Sampling was carried out from 1997 to 2001 at three sites in the Upper Lake, each representing a different type of littoral zone. Site "South" is located on the southern shoreline of the lake (Fig. 1) and has a narrow (50 m) shelf-like littoral zone. Large stones and boulders providing distinct interstitial shelters characterize the uppermost shoreline, which is flooded only during high-water conditions from May to September. In contrast, interstitial shelter is sparse during the rest of the year, especially in winter when the substratum is mainly characterized by sand and pebbles interspersed with only slightly larger stones. Macrophyte coverage during summer is sparse, mainly dominated by *Potamogeton pectinatus*, *Potamogeton perfoliatus*, and *Chara* spp., and only present in a zone between 3 and 5 m deep. The site is exposed to winds from the northeast that, however, blow only a few days a year. During the remainder of the year, a landward ridge covering the major part of this shoreline protects the site.

Site "North-1" is located on the northwestern shoreline (Fig. 1) and has a lakeward extent of 200–300 m. The substratum of most of the area, including the uppermost shoreline, is characterized by gravel and pebbles providing a heterogeneous, but not as well-developed, interstitial coverage as the southern site. Soft sediments are present only in the deeper areas with sparse macrophyte coverage (mainly *P. pectinatus* and *Chara* spp.) during summer. The site is exposed to winds from the southeast, but owing to its geographical position within the fjord-like extension of the Upper Lake, "Lake Ueberlingen" (Fig. 1), the site is protected against strong wind and wave action year round.

The third site, "North-2", is also located on the northern shoreline about 35 km east of North-1 (Fig. 1). This site has a littoral zone with a lakeward extension of 500–700 m. The substratum is composed of soft sediment with little rocky stone cover. Macrophytes are present in the deeper parts during summer even though the total coverage is sparse, with *P. pectinatus* presently the dominant species. This site has been subject to a dramatic decrease in macrophyte vegetation cover, a very dense population of *P. pectinatus* in the early 1990s being now reduced to only a few plants. Owing to its unprotected location, this site is the most exposed to winds coming from all directions.

Sampling was carried out at the three sites according to three temporal factors: year (from 1997 to 2001), month (from March to December), and time of day (day or twilight). The sampling plan applied in this study therefore consisted of four factors: site, year, month, and time of day. Sampling was standardized at all three sites, with beach

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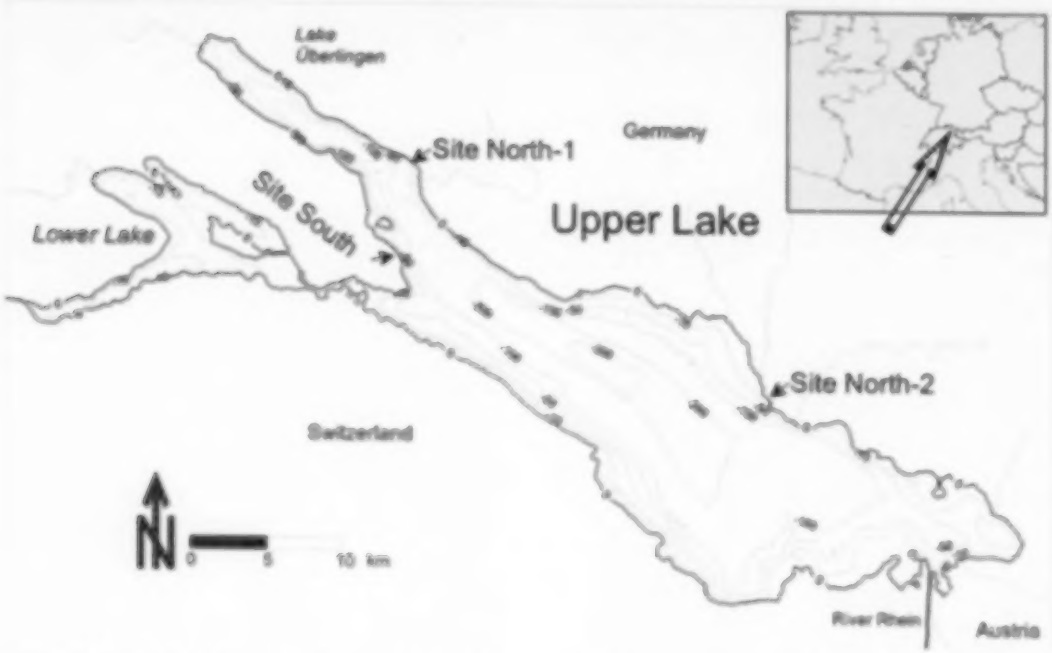
Fig. 1. Study area and sampling sites in Lake Constance situated at the boundary of Austria, Germany, and Switzerland.



seines (length 16 m, height 1 m, mesh size 4 mm (bar mesh)) being hauled from 1 m water depth (height of the seine) to the shoreline. On each sampling occasion, three replicate samples were collected so that for each month, a total of 18 samples (3 sites × 2 times (day and twilight) × 3 replicates) were taken. However, for various reasons (e.g., an extreme high water level in May 1999), this sampling schedule was not strictly adhered to over the entire 5 years. Nevertheless, because missing samples were few and occurred simultaneously at all sites, the number of samples remained comparable across sites. All fish caught within a seine haul were immediately killed with 2-phenoxyethanol solution, or alternatively clove oil, and stored on ice. In the laboratory, all fish were identified to species and counted.

Statistical treatment and data modeling

Community structure

First, a species abundance data set was arranged as a matrix of 184 rows (i.e., the samples) and 25 columns (i.e., the species). Each cell represented the mean number of individuals of a species caught during one sampling event based on the three replicates (e.g., 1997, July, site North-1, twilight). To have an overview of community structure according to species' mean abundance in the samples, we performed a hierarchical cluster analysis with the Ward linkage method.

SOM

Each of the 184 samples of the input data set can be considered as a vector of 25 dimensions in the n-dimensional space $\boldsymbol{R}^n$. The aim of the SOM is to project these samples on a two-dimensional space (i.e., the Kohonen map) preserving the neighborhood so that samples with close species abundances should be mapped together on the grid, and conversely, samples with very different species abundances should be mapped far from each others (full details on the method can be found in Ripley 1996; Lek and Guégan 2000; Kohonen 2001). The form of the Kohonen map is a hexagonal lattice because it does not favor horizontal or vertical directions as much as the rectangular array (Kohonen 2001). The SOM consists of two layers, i.e., one input layer and one output layer, connected with computational weights (i.e., connection intensities). The input layer is connected to each vector of the data set and the output layer corresponds to the Kohonen map. The input layer used for the present model consisted of 184 neurons, while the output layer consisted of 35 neurons organized on an array with seven cell-rows and five cell-columns. This configuration of the output layer was chosen because of the clarity of the representation obtained compared with other configurations.

The learning process of the SOM is as follows. Each neuron of the output layer comprised one virtual unit. The virtual units of the Kohonen map are initialized with random sample units drawn from the input data set. Then, the virtual units are updated in an iterative way: a sample unit is randomly chosen in the input data set. The Euclidian distance between this sample and every virtual unit is computed. The virtual unit that has the minimum distance with the input unit is selected as the best matching unit. For the best matching unit and its neighbors, the weight vectors are updated by the SOM learning rule. This results in training the network to classify the input vectors by the weight vectors that they are closest to.

The virtual assemblages associated with the Kohonen map were visualized using a grey scale.

The SOM was performed using the toolbox developed by Alhoniemi et al. (2003) for Matlab.

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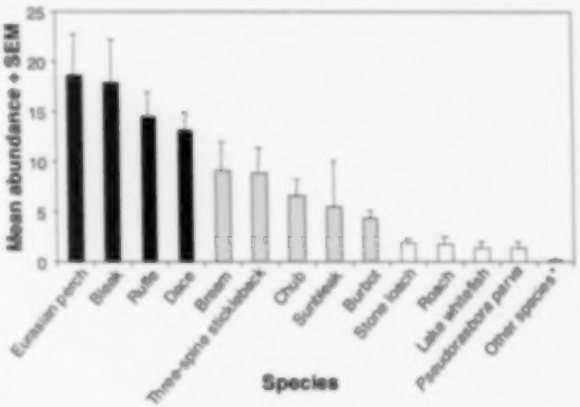
Table 1. Common and scientific names of the species composing the littoral fish community of Lake Constance in the present study.

Common name	Scientific name
Eurasian perch	*Perca fluviatilis*
Bleak	*Alburnus alburnus*
Ruffe	*Gymnocephalus cernuus*
Dace	*Leuciscus leuciscus*
Bream	*Abramis brama*
Threespine stickleback	*Gasterosteus aculeatus*
Chub	*Leuciscus cephalus*
Sunbleak	*Leucaspius delineatus*
Burbot	*Lota lota*
Stone loach	*Barbatula barbatula*
Roach	*Rutilus rutilus*
Lake whitefish	*Coregonus lavaretus*
Stone moroko	*Pseudorasbora parva*
Common carp	*Cyprinus carpio*
Tench	*Tinca tinca*
Pikeperch	*Stizostedion lucioperca*
Rudd	*Scardinius erythrophthalmus*
European eel	*Anguilla anguilla*
Brown trout	*Salmo trutta*
Grayling	*Thymallus thymallus*
Prussian carp	*Carassius gibelio*
Northern pike	*Esox lucius*
Common barbel	*Barbus barbus*
Sculpin	*Cottus gobio*
Crucian carp	*Carassius carassius*

Fig. 2. Mean abundance and SEM for each species caught by seining the littoral zone of Lake Constance from 1997 to 2001. The different groups were separated according to a hierarchical cluster analysis with the Ward linkage method. Solid bars, species with a high level of abundance; shaded bars, species with an intermediate level of abundance; open bars, species with a low level of abundance. *Other species: common carp (*Cyprinus carpio*), tench (*Tinca tinca*), pikeperch (*Stizostedion lucioperca*), rudd (*Scardinius erythrophthalmus*), European eel (*Anguilla anguilla*), brown trout (*Salmo trutta*), grayling (*Thymallus thymallus*), Prussian carp (*Carassius gibelio*), northern pike (*Esox lucius*), common barbel (*Barbus barbus*), sculpin (*Cottus gobio*), and crucian carp (*Carassius carassius*).



Definition of SOM clusters

The weight vectors of each virtual unit were submitted to a hierarchical cluster analysis with the Ward linkage method to detect clusters on the trained SOM map. To associate each species to one cluster, we calculated the sum of the estimated abundances generated by the SOM for each species in the cells of each cluster and then assigned the species to the group with the highest value.

Influence of time and space on the fish community

The last step was to assess the relationships between the fish community associated with each cluster and the factors of the sampling plan (year, month, site, and time of day), which were not used in the SOM learning process. To do so, we calculated for each cluster the relative contribution of each modality (e.g., July) of a certain factor (e.g., month) to the total number of modalities of the factor. Then, we tested for the H_0 that no significant differences were present among these relative values using a test for proportions based on the likelihood ratio χ^2 statistics (Sachs 1997). The SOM was computed using MATLAB® software (The MathWorks, Inc., Natick, Massachusetts). All subsequent proportion tests were done in Microsoft Excel® based on the calculation procedures of Sachs (1997).

Community structure

A total of 57 557 fish belonging to 25 species were caught during the 5-year sampling program (Table 1). According to a cluster analysis, four of these species were found with high average ± SEM abundances over the entire sampling period (from 18.7 ± 4.1 to 13.2 ± 1.8 individuals per replicate), five species were found at an intermediate level of abundance (from 9.1 ± 2.8 to 4.4 ± 0.6 individuals per replicate), and 16 species were found in low abundances (<2.0 ± 0.3 individuals per replicate) (Fig. 2).

SOM

According to the cluster analysis used to detect the cluster boundaries on the trained SOM map, four clusters were defined, which encompassed 74, 49, 29, and 32 samples, respectively.

The tests for proportions revealed significant patterns for the four factors of the sampling design. Considering cluster 1, the samples were significantly related to the factors month ($G = 49.4$, df = 9, $p < 0.05$) and time of day ($G = 7.7$, df = 1, $p < 0.05$). This cluster grouped together samples from March and April with samples from November and December, i.e., winter and early spring samples. Considering time of day, cluster 1 included significantly more day than twilight samples (Fig. 3*b*). The corresponding fish community was made up of sculpin (*Cottus gobio*), brown trout (*Salmo trutta*), and grayling (*Thymallus thymallus*) in decreasing order of the sum of their estimated abundance in the cluster (Fig. 4).

The samples within cluster 2 were significantly related to month ($G = 78.1$, df = 9, $p < 0.05$) and time of day ($G = 6.0$, df = 1, $p < 0.05$). As for cluster 1, this cluster included significantly more day than twilight samples but corresponded to samples taken from May through August, i.e., late spring and summer samples (Fig. 3*b*). Fish species typically associated with this cluster were crucian carp (*Carassius carassius*),

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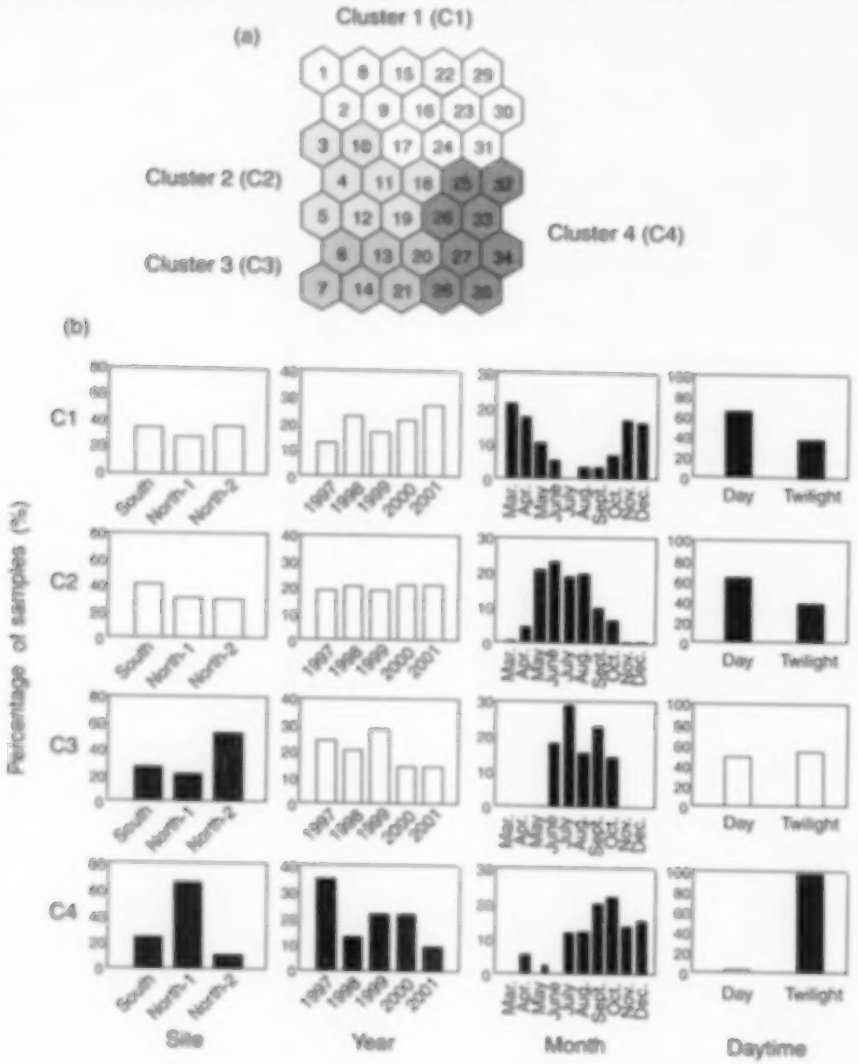

Fig. 3. (*a*) Two-dimensional SOM. Each cell represents one neuron, which groups together several samples. The clusters (C1–C4) of the SOM defined by the hierarchical analysis are illustrated with different shades of gray. (*b*) Relative contribution of each modality of a certain factor to the total number of modalities of the factor. Solid bars, significant relationships ($p < 0.05$).

lake whitefish (*Coregonus lavaretus*), dace (*Leuciscus leuciscus*), and northern pike (*Esox lucius*) (Fig. 4).

The samples grouped in cluster 3 were significantly related to month ($G = 102.9$, df = 9, $p < 0.05$) and to sampling site ($G = 16.9$, df = 2, $p < 0.05$). They mainly represented the summer months, i.e., June through September, as well as samples taken at site North-2 (Fig. 3*b*). The corresponding fish community was composed of common carp (*Cyprinus carpio*), pikeperch (*Stizostedion lucioperca*), Prussian carp (*Carassius gibelio*), bream (*Abramis brama*), roach (*Rutilus rutilus*), Eurasian perch (*Perca fluviatilis*), and bleak (*Alburnus alburnus*) (Fig. 4).

Finally, the samples composing cluster 4 were significantly linked to every factor of the sampling design: month ($G = 61.4$, df = 9, $p < 0.05$), time of day ($G = 88.4$, df = 1, $p < 0.05$), site ($G = 49.6$, df = 2, $p < 0.05$), and year ($G = 19.7$, df = 4, $p < 0.05$). In this cluster, samples from July through December were overrepresented (especially September and October, i.e., partly autumn), as well as samples taken from site North-1 and twilight samples. The samples grouped within this cluster also showed a significant negative trend over the sampling period as a whole, with the highest proportion value in 1997 and the lowest in 2001 (Fig. 3*b*). The associated fish community comprised sunbleak (*Leucaspius delineatus*), burbot (*Lota lota*), stone moroko (*Pseudorasbora parva*), stone loach (*Barbatula barbatula*), tench (*Tinca tinca*), common barbel (*Barbus barbus*), chub (*Leuciscus cephalus*), European eel (*Anguilla anguilla*), rudd

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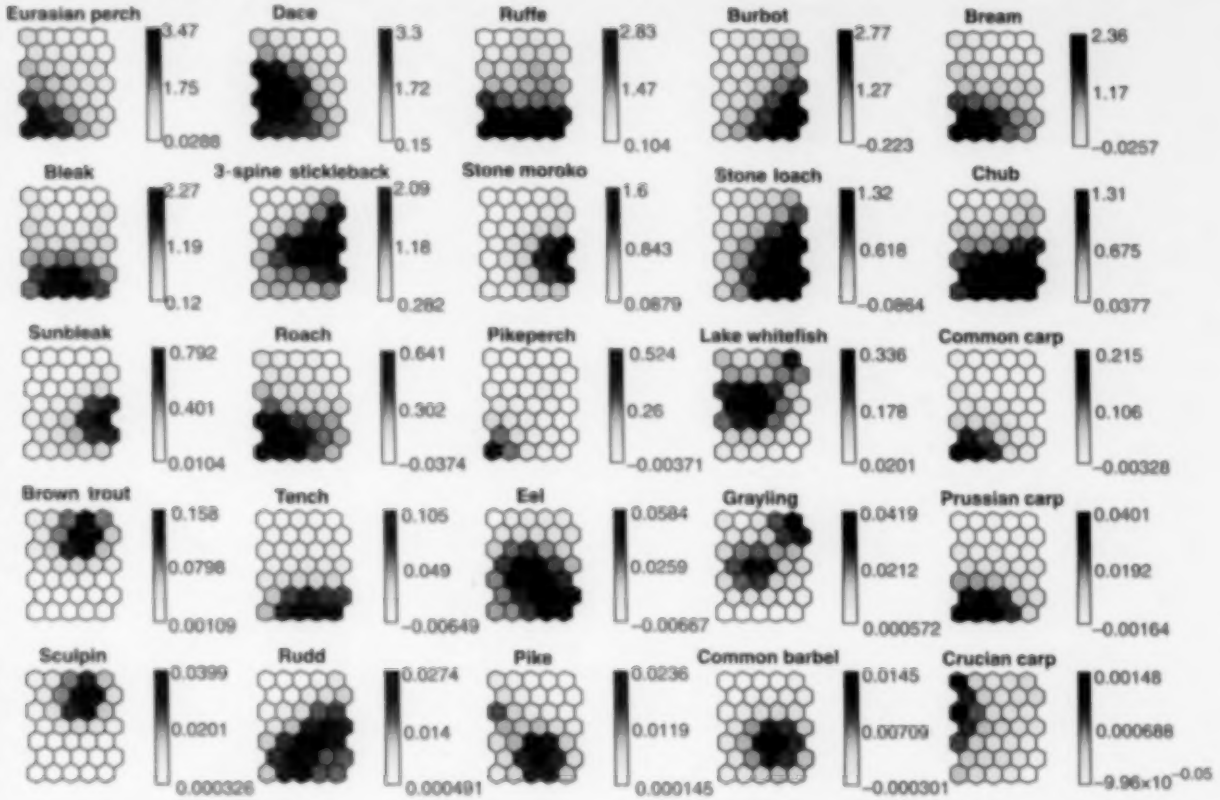
Fig. 4. Estimated abundance of each species in each cell of the SOM. Species are presented in decreasing order of maximal estimated abundance (from top left to bottom right).



(*Scardinius erythrophthalmus*), threespine stickleback (*Gasterosteus aculeatus*), and ruffe (*Gymnocephalus cernuus*) (Fig. 4).

Discussion

From a total of 33 fish species in Lake Constance (Eckmann and Rösch 1998), 25 species were found in the littoral zone during this 5-year study (i.e., more than 75%). This high percentage confirms the important role played by littoral areas for fish populations and assemblages (Crowder and Cooper 1982; Werner et al. 1983; Lobb and Orth 1991). Compared with the previous work by Fischer and Eckmann (1997*b*) on Lake Constance, more species were found in the present study (25 versus 19). This difference is probably attributable to a different sampling strategy, since more sites were sampled by Fischer and Eckmann (1997*b*) but over a shorter time period and mainly during daytime.

According to the tests for proportions, every factor of the sampling design appeared to significantly influence one or several of the fish assemblages isolated by the SOM. The clearest patterns observed were those related to the annual cycle (factor month), which has to be related to the annual littoral thermal regime of the lake and to the species' thermal preferences. The winter to early spring assemblage revealed by the SOM corresponded with sculpin, brown trout, and grayling, which are usually classified as cold stenotherms and characteristic of cold-water areas with high oxygen content (e.g., Pickering 1981; Matthews 1998; Reyjol et al. 2001*b*). The late spring to summer community comprised species like lake whitefish and dace, which are also cold-water species, although they tolerate warmer water temperatures (Mann 1996). Finally, the summer and autumn communities clearly gathered typical warmwater species, e.g., bream, roach, Eurasian perch, bleak, common carp, Prussian carp, tench, and ruffe (Pickering 1981; Mann 1996). Studies describing such a monthly pattern of succession for European freshwater species in lakes are only sparse (but see Fischer and Eckmann 1997*a*, 1997*b*). Most of our knowledge on the distribution patterns of fish according to their thermal preferences comes from the spatial pattern of succession observable from upstream to downstream of rivers as temperature increases (for details, see Matthews 1998). It is most striking that SOM revealed temporal patterns of succession in the littoral fish community in Lake Constance that are very close to the spatial pattern of succession in the fish community in streams. This might become especially important for future studies on littoral fish communities of large lakes. Compared with stream ecology, our knowledge on littoral fish communities in large lakes and especially on the factors determining the temporal and spatial distribution of fish in certain habitats is still restricted. Our SOM analysis provided some evidence that the mechanisms determining

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the distribution of fish in streams and lakes might be similar even though expressed in different scales (spatial scale in streams versus a temporal scale in lakes). This, however, would allow the transfer of ecological concepts developed in stream ecology to large lake ecosystems and therefore provides the opportunity to enhance our restricted knowledge on large lake ecosystems in general. The fact that our modified SOM analysis revealed these patterns in Lake Constance is all the more precious because Lake Constance is the second largest prealpine lake in Europe and therefore can be well assumed as a most relevant model system for many other temperate large lake ecosystems on the Northern Hemisphere.

The second result of the SOM was the clear gradient from a strictly daytime community notably constituted by sculpin, brown trout, grayling, dace, and lake whitefish to a twilight community gathering sunbleak, burbot, stone moroko, stone loach, chub, threespine stickleback, and ruffe. The results obtained for the daytime community associated with cluster 1 have to be considered carefully because of the very low catches recorded for the species over the entire sampling period (<2.0 ± 0.3 individuals per replicate). On the contrary, the other results fit with previous studies on fish nycthemeral habits, especially for burbot and stone loach, previously described as typically crepuscular species hiding within substratum interstices during daytime (Burdeyron and Buisson 1982; Fischer 2000). The twilight preference of the other species is much less documented in the literature and has to be considered with interest. The fact that SOM revealed a gradient instead of a simple dichotomy (daytime versus crepuscular species) in the present study is most interesting. This would suggest that a strict nycthemeral preference is more the exception than the rule and only expressed in few species related to species-specific physiological and probably genetic adaptation (e.g., burbot and stone loach). The results suggest that most other species are probably more plastic with regard to this biological trait allowing more a flexible and species-specific pattern of habitat partitioning with regard to daytime. This is especially true with regard to dace, which was found in the present study as a typical daytime species, while Clough and Ladle (1997) previously found it as a nocturnal species in the River Frome. Diel activity patterns especially in juvenile fish are generally assumed as a biological trait between the need for somatic growth to increase individual fitness and the need for refuge against predation by larger piscivorous daytime predators such as pike (Bain et al. 1988; Ruiz et al. 1993; Gibson and Robb 1996). Assuming optimal foraging, to optimize a behavior within these two conflicting demands, a highly plastic behavior with respect to diel activity patterns is much more likely to exist (in an evolutionary way) than a strict bimodal daytime–nocturnal decision with little chance for modulation. More than 90% of the fish in the present study were juveniles. Such a plastic behavior is especially important during the first year of life, during which fish have to grow as fast as possible to limit predation pressure and after which resource allocation progressively switches to maintenance and reproduction (Wootton 1998).

Considering sampling site, the SOM clearly isolated two distinct assemblages. The first one associated site North-2 with common carp, pikeperch, Prussian carp, bream, rouch, Eurasian perch, and bleak, while the second one gathered sunbleak, burbot, stone moroko, stone loach, chub, threespine stickleback, and ruffe within site North-1. Compared with site South, which has a comparatively narrow shelf-littoral area, both North sites are characterized by a low slope and therefore an extended littoral area. Site North-1, however, differs from site North-2 by its very heterogeneous substratum composed of large stones and cobbles. This substratum type provides large interstices where crepuscular benthic species such as burbot and stone loach can shelter. On the contrary, site North-2 is characterized by fine sediments with dense stand of submerged macrophytes, which are know to be suitable habitats for species like bream, rouch, and Eurasian perch (Hinch and Collins 1993; Fischer and Eckmann 1997*a*, 1997*b*). Habitat partitioning in lakes has especially been studied at the scale of the study site, where some species have been found to colonize shorelines, while others colonize substratum or more or less deep areas (Pierce et al. 1994; Fischer and Eckmann 1997*a*, 1997*b*). The present study gives an example of how habitat can be partitioned within different sampling sites and therefore at the scale of the whole lake, which probably has an influence on the coexistence of different species populations within a lake. Even if only three sampling sites were considered in the present study, a similar habitat partitioning can be found in Fischer and Eckmann (1997*a*, 1997*b*) where more sites were considered. This testifies to the necessity of maintaining habitat diversity in littoral zones of lakes to promote fish diversity.

Finally, the last trend revealed by the analyses was a significant decreasing proportion of samples in which sunbleak, burbot, stone moroko, stone loach, chub, threespine stickleback, and ruffe were present from 1997 to 2001. While chub and ruffe dominated the cyprinid and percid community during the 90s of the last century (Fischer and Eckmann 1997*b*), recent studies on Lake Constance showed that these species underwent a strong decrease in abundance over the last years. This decline probably reflects the significant change in the trophic status of Lake Constance from a mesotrophic to eutrophic lake in the late 1980s (i.e., >80 $\mu g\ P{\cdot}L^{-1}$) to an oligotrophic lake at present (i.e., <12 $\mu g\ P{\cdot}L^{-1}$). In contrast with the process of eutrophication, for which effects like an increase in total catches in cyprinids together with the decrease in percids, salmonids, and coregonids are well documented, the effects of the reoligotrophication process on lake fish communities are almost entirely lacking. Assuming a simple reversal of the process and effects of eutrophication can hardly be assumed, especially when new species have been introduced into the system. In Lake Constance, ruffe was found for the first time in 1987, and since then, its population increased dramatically until 1995 (Rösch and Schmid 1996). Our SOM results now revealed that for Lake Constance, among percids, only ruffe but not perch was associated with a decreasing trend. This indicates that ruffe might be much more affected by the long-term changes in the tropic status compared with Eurasian perch. If a higher or earlier sensitivity to the reoligotrophication process of ruffe compared with Eurasian perch would be confirmed, this species could be considered as a relevant biological sentinel to follow the reoligotrophication process in lakes. The theoretical following step for the littoral fish

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community of Lake Constance in the next years should be the increase of species which were dominant during the first half of the last century when Lake Constance was on a trophic state similar to nowadays. Exactly this is observed today with a strong increase in bleak and with lake whitefish becoming again more important in the commercial fishery. Especially the latter development, i.e., the refreshment of lake whitefish, is most interesting, not only in terms of importance to the local fishermen but also in terms of intraspecific interactions with ruffe. Rösch and Schmid (1996) showed that in Lake Constance during the spawning season of lake whitefish, ruffe almost entirely switches to lake whitefish eggs as a main prey. If long-term changes in the trophic status of the lake selectively negatively affect ruffe abundance, the decrease in the nutrition load of the lake not only positively affects lake whitefish recruitment itself by an increase in egg survival in the deep profundal areas. It also may indirectly affect stock size by a decrease in one of its main predators on the most critical ontogenetic stage of development.

In addition to using SOM on these data, we did try a number of more traditional approaches. We initially ran a PCA on instrumental variables (PCAiv), a method that was developed for focusing on the different effects of factors in sampling designs (Sabatier et al. 1989; Lebreton et al. 1991). The results from this method were difficult to interpret, probably owing to the large number of factors used here. A classical PCA was also used on the data to cluster the samples to investigate the effects of the different factors, but the results from this analysis were also difficult to interpret.

Multivariate analysis of variance (MANOVA) seemed to be a natural contender here and with this approach, we found that the results were similar to those from SOM with regard to the effects of month and daytime but there were major differences between the two methods concerning the factors site and year. That is, significant associations with site South were found for some species with MANOVA, and SOM only detected the interannual decreasing trend observed for burbot, ruffe, and chub from 1997 to 2001, while other relationships were found with MANOVA.

In MANOVA, each fish species was related to each of the factors and the grouping of species with similar ecological responses was made after the fact. On the other hand, with the SOM approach, species were clustered first and then the significance of trends for the sampling design factors was tested using χ^2 statistics. Despite these differences in approach and while MANOVA detected more significant relationships than SOM did, a number of the trends revealed by the SOM approach were confirmed by MANOVA, testifying to their robustness. While using more than one kind of statistical model for the same data can identify the more robust findings, we believe that the SOM method used here allows for a very interesting view of the whole community organization and functioning with respect to space and time (superimposition of Figs. 3 and 4), which as far as we know has never been documented for such a complex sampling design as used here.

In conclusion, the SOM methodology used in this study clearly separated distinct communities according to site, year, month, and time of day. The explicit, simultaneous consideration of spatial and temporal variation allowed a valuable interpretation of temporal and spatial patterns, as previously suggested by Resh and Rosenberg (1989). Consequently, SOM appears to be a very promising approach to analyzing large data sets built according to complex sampling designs. With regard to lake functioning in general, this study clearly illustrated how habitat utilization is partitioned by fish both spatially (i.e., among sites) and temporally (i.e., between daytime and twilight), which highlighted the complexity of fish community organization and functioning in the littoral zones of lakes.

Acknowledgements

We thank all of our colleagues and student assistants for their great help in the field over the 5-year sampling period and Myriam Schmid and Pia Mahler for their work in species identification and counting. We also thank Olivier Beauchard and Pierre S.G. Bady for their very fruitful help in multivariate statistics and four anonymous reviewers for their improvements to this manuscript. This work was supported by the German Research Foundation within the Special Collaborative Project SFB 454 "Littoral of Lake Constance".

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Influence of acidic to basic water pH and natural organic matter on aluminum accumulation by gills of rainbow trout (*Oncorhynchus mykiss*)

Anna R. Winter, Joel W. Nichols, and Richard C. Playle

Abstract: Juvenile rainbow trout (*Oncorhynchus mykiss*) (~0.6 g) were exposed to 3 μmol Al·L^{-1} in ion-poor water adjusted to pH 4–10 in the absence or presence of natural organic matter (NOM). Aluminum accumulation by trout gills was highest at pH 6–8, there was moderate Al accumulation by trout gills at pH 5 and 9, and trout at pH 4 and 10 did not accumulate any Al on their gills. NOM at 5 mg C·L^{-1} eliminated Al accumulation by trout gills at all water pHs. These results are explained by NOM complexing Al and keeping Al in solution but off the gills, by H^+ competition with Al^{3+} at low pH, by poor binding of the $Al(OH)_4^-$ anion to negatively charged gills at high pH, and by polymerization and precipitation of Al onto the gills at intermediate water pH, especially if water pH in the gill microenvironment is considered. Increased fish mortality at pH 10 in the presence of NOM is explained by the indirect effect of NOM tying up the limited amount of Ca in the ion-poor water.

Résumé : Nous avons exposé de jeunes truites arc-en-ciel (*Oncorhynchus mykiss*) (~0.6 g) à 3 μmol Al·L^{-1} dans de l'eau à faible teneur en ions à des pH ajustés de 4 à 10, en l'absence ou en présence de matière organique naturelle (NOM). L'accumulation d'aluminium par les branchies de truites est maximale de pH 6 à pH 8, elle est moyenne à pH 5 et à pH 9 et elle est nulle à pH 4 et à pH 10. Une concentration de 5 mg C·L^{-1} de NOM élimine l'accumulation d'Al par les branchies de truites à tous les pH de l'eau. Ces résultats s'expliquent par le fait que NOM forme des complexes avec Al, le maintient en solution et l'éloigne des branchies; de plus, l'ion H^+ fait compétition avec Al^{3+} à pH bas, l'anion $Al(OH)_4^-$ se lie mal aux branchies qui ont une charge négative à pH élevé et Al se polymérise et précipite sur les branchies aux pH intermédiaires de l'eau, particulièrement si l'on considère le pH dans le microenvironnement de la branchie. La mortalité accrue des poissons à pH 10 en présence de NOM s'explique par l'effet indirect de NOM qui se lie à la quantité réduite de Ca dans l'eau pauvre en ions.

[Traduit par la Rédaction]

Introduction

Aluminum is the most common metal in the earth's crust, has no known biological function, and takes many different physicochemical forms that vary in toxicity to aquatic biota (for recent reviews, see Sparling and Lowe (1996) and Gensemer and Playle (1999)). It is generally agreed that inorganic monomeric Al species are the most toxic and they include, from acidic to basic conditions, Al^{3+}, $AlOH^{2+}$, $Al(OH)_2^+$, $Al(OH)_3$, and $Al(OH)_4^-$. Aluminum solubility is lowest between pH 6 and 8, where Al polymerizes and precipitates out of solution. Aluminum solubility increases in acidic or alkaline conditions, at lower temperatures, or with the addition of complexing ligands (e.g., Sparling and Lowe 1996; Peuranen et al. 2003). Aqueous Al is the main toxicant affecting fish in acidic waters and acts as a gill toxicant to fish, causing ionoregulatory and respiratory effects or a mixture of these two effects (Neville 1985; reviewed by Gensemer and Playle 1999; also see Peuranen et al. 2003).

When the pH of an acidic water system is rapidly increased, Al solubility decreases, resulting in an increase in acute Al toxicity as Al polymerizes and precipitates onto the surface of the gills of fish (e.g., in mixing zones of acidic and neutral water or during liming) (e.g., Poléo 1995; Exley et al. 1996; Kroglund et al. 2001). Natural organic matter (NOM) in water decreases Al toxicity by acting as a complexing ligand: Al is bound by NOM and is kept in solution instead of accumulating on fish gills (Roy and Campbell 1997; Gensemer and Playle 1999; Peuranen et al. 2003). Playle and Wood (1989*a*, 1989*b*) demonstrated that water pH near the gills of adult rainbow trout (*Oncorhynchus mykiss*) is different than the bulk solution pH, and therefore, Al speciation and Al solubility are altered near the gills. Acidic, poorly buffered water is made more basic as it passes

Received 6 July 2004. Accepted 3 February 2005. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on 22 September 2005.
J18209

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[2]Regrettably, Dr. Richard Playle passed away in July of this year. He was an excellent scientist, teacher, mentor, and friend. He will be sorely missed — A.R. Winter and J.W. Nichols.

 doi: 10.1139/F05-063 © 2005 NRC Canada

over the gills by the release of ammonia (NH_3), which consumes H^+ to form ammonium (NH_4^+), and basic water is made more acidic by carbon dioxide release at the gills, which generates H^+ as bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) are formed.

Although much is known about the effects of Al on fish in acidic environments, interactions of Al with fish in alkaline environments is much less extensively documented (Heming and Blumhagen 1988; Gundersen et al. 1994; Poléo and Hytterød 2003). A model of Al–gill interactions assumes that Al^{3+} binds to negatively charged ligands on the gill surface and then has toxic effects on or in the gills and that neutral $Al(OH)_3$ precipitates onto fish gills as gibbsite (Gensemer and Playle 1999). The model assumes that the aluminate anion $Al(OH)_4^-$ does not bind to negatively charged gills, and indeed, this species of Al does not appear to be very toxic to fish (Poléo and Hytterød 2003). This Al–gill model is a type of biotic ligand model (Paquin et al. 2002; Playle 2004), and the model was used to simulate Al interactions with gills of fish at pH 4–7 (Gensemer and Playle 1999) and was applied to acidic, NOM-rich blackwater (Wood et al. 2003), but Al–gill interactions have not been modelled for higher water pHs.

We investigated Al binding to gills of rainbow trout in acidic to basic water in the presence and absence of NOM to better determine the mechanism of Al toxicity in fish, especially at high pH. The Al concentration used (3 μmol·L^{-1}) was within the critical concentration range of inorganic Al to various fish species (e.g., Lydersen and Löfgren 2002) and is a concentration of Al that can be detected and avoided by rainbow trout (Exley 2000).

Materials and methods

Experimental fish

Juvenile rainbow trout (0.3–1.0 g, mean = 0.6 g) were obtained from Rainbow Springs trout farm in Thamesford, Ontario. Fish were housed in aerated 1:1 well water and ion-poor (soft) water and were fed ground HiPro Alevin Corey Parr fish food (Corey Feed Mills Ltd., Fredericton, New Brunswick). Fish were acclimated to ion-poor water for at least 1 week before an experiment, supplied by a reverse osmosis unit (Series E Reverse Osmosis System, Culligan of Canada, Mississauga, Ontario); a Ca drip was added to maintain fish health. Water chemistry of the acclimation water was approximately 16 μmol Ca·L^{-1}, 900 μmol Na·L^{-1}, and 180 μmol Cl·L^{-1}, pH 6.8, at 17 °C. Fish were not fed for 24 h before an experiment. The Canadian Council of Animal Care approved housing and experiments.

Experiments at pH 4–10

Ten trout were randomly assigned to each of eight polyethylene buckets containing nominal 3 μmol Al·L^{-1} (as $Al_2(SO_4)_3{\cdot}16H_2O$; AnalR reagent) in 10 L of ion-poor water. The buckets were manually adjusted to nominal pH 4, 5, 6, 7, 8, 9, and 10 using dilute HNO_3 or KOH. There was one control exposure, which was pH 7 with no added Al. Five fish per exposure were sampled at 3 h and, if available, at 48 h. Fish were not fed to prevent complications from Al uptake from feeding or loss of Al to feces. Water pH was monitored and adjusted manually every hour initially to keep pH within about 0.2 pH unit of nominal pH values of 4–7 and about 0.5 pH unit of nominal pH values of 8–10. Larger pH excursions occurred with less frequent pH adjustment as the experiments progressed (e.g., overnight), so the 3-h results with greater pH control are emphasized.

This experiment was repeated 1 week later with the addition of Suwannee River NOM collected by us. The Suwannee River drains the Okeefenokee Swamp in southern Georgia, USA, and Suwannee River NOM is often used in metal–NOM studies (e.g., Larrivee et al. 2003). River water was filtered (1-μm pore size), concentrated about 40-fold using a stainless steel portable reverse osmosis unit (Limnological Research Corporation, Kelowna, British Columbia), and then treated with a H^+ exchange resin to pH <2. Nineteen millilitres of the concentrated NOM solution was added to the 10-L exposure containers to reach nominal total organic carbon (TOC) concentrations of 5 mg C·L^{-1}. Greater technical detail may be found in Schwartz et al. (2004) and Luider et al. (2004).

Experiments at pH 10

This experiment was run to help explain decreased survival of trout in alkaline conditions when Suwannee River NOM was added. Ten fish were added to each of six buckets containing 10 L of ion-poor water. The treatments were as follows: control (pH 7, no added Al), pH 10 alone, pH 10 with 5 mg TOC·L^{-1}, pH 10 with 5 mg TOC·L^{-1} plus 3 μmol Al·L^{-1}, pH 10 plus 3 μmol Al·L^{-1}, and pH 10 with 5 mg TOC·L^{-1} plus 450 μmol Ca·L^{-1}. Fish mortality and water pH were monitored every hour for 10 h.

Fish sampling plus gill and water analysis

Fish were killed by a blow to the head and both sets of gill arches were removed. Gills were rinsed in 100 mL of ultrapure water for 10 s to remove loosely bound Al and were placed into 1.5-mL microcentrifuge tubes. Gills were digested by mixing each gill sample with five times its weight in 1 mol·L^{-1} HNO_3 and heating to 80 °C for 4 h. After digestion, each sample was vortexed and allowed to settle. The supernatant of each sample was diluted 10 times by placing 100 μL of supernatant into 900 μL of ultrapure water in new 1.5-mL microcentrifuge tubes.

Water samples in all experiments were taken at 1, 24, and 48 h and analyzed for Al, TOC, Ca, Na, and Cl. Filtered Al and C samples were taken using 0.45-μm Millex-HV filters (Millipore, Bedford, Massachusetts). Each filter was rinsed with 40 mL of sample water before sample collection. Samples for Al, Ca, and Na analysis were placed into 7-mL scintilation vials and acidified with one drop of 16 mol·L^{-1} HNO_3; similar samples for Cl analysis were not acidified. Samples for TOC analysis were placed into borosilicate vials that had been combusted for 2 h at 450 °C.

Gill and water samples were analyzed for Al by graphite furnace atomic absorption spectrophotometry (GTA100 atomizer, SpectrAA 880, N_2 gas; Varian, Mississauga, Ontario) using standards prepared from a Fisher reference Al solution run every 25 samples. Sample injection volume was 10 μL, and the furnace operating procedures (as provided by Varian) were as follows: 5 s at 85 °C, 40 s at 95 °C, 10 s at 120 °C, 8 s at 1000 °C, and 4.8 s at 2500 °C. During the first 2.8 s of the last step, absorbance was measured at 309.3 nm.

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Detection limits were about 0.002 μmol Al·L^{-1} but are reported to 0.01 μmol Al·L^{-1} for water samples.

Water pH was measured using a PHM82 standard pH meter (Bach-Simpson Limited, London, Ontario) and a GK2401C combined pH electrode. Water Ca and Na were measured by flame analysis using a Varian SpectrAA 880. Chloride analysis was done by mixing 1 mL of water with 1 mL of chloride reagent (Biopacific Diagnostics Inc., North Vancouver, British Columbia) and then measuring absorbance at 460 nm using a spectrophotometer (Spectronic 301, Milton Roy, Rochester, N.Y). TOC was measured with a Shimadzu TOC 5050A analyzer (Schimadzu Corp., Kyoto, Japan) after sample sparging with N_2 gas to remove inorganic C.

Water pH in the gill microenvironment was estimated from measured water pH and data originally generated for rainbow trout by Playle and Wood (1989*a*). In that study, ion-poor water was drawn from near the branchial surface of adult rainbow trout (~280 g) using opercular catheters, and measured pH of the expired water was compared with inspired water pH. Concentrations of oxygen, carbon dioxide, NH_3, and titratable base were also measured in expired and inspired water (Playle and Wood 1989*a*). Similar differences between expired and inspired water pH were seen for small (~7 g) fathead minnows (*Pimephales promelas*) (Playle et al. 1992; trout and minnow data presented together in Playle 1998).

Statistical analysis

Gill Al concentrations were means of five or four trout ± 1 SE of the mean. Gill Al values from exposures to Al in the absence and presence of NOM at a particular water pH, done 1 week apart, were compared using Student *t* tests. At pH 7, the gill Al values of Al-exposed trout were also compared with control values (no added Al) from the two experiments ($n = 10$) using one-way analysis of variance followed by Tukey's multiple comparison test.

Results

Influence of water pH and NOM on Al accumulation by rainbow trout gills

When rainbow trout were exposed to Al in very acidic water to very alkaline water, Al accumulation by their gills in 3 h was highest at pH 6.2, 7.0, and 8.0, and there was moderate Al accumulation at pH 4.9 and 8.8 (Fig. 1*a*). At pH 7, the accumulation in gills of Al-exposed trout was significantly higher ($P < 0.001$) than the pH 7 control values of about 28 nmol·g wet weight^{-1} (Table 1). Trout exposed to Al at pH 4.1 and 9.8 did not accumulate any Al on their gills (e.g., gill Al concentrations at pH 4.1 and 9.8 were similar to the 3-h control values). Trout in these two extreme pHs did not survive past 24 h. Gill Al concentrations for surviving trout sampled at 48 h are also given (Table 1); these values were higher than at 3 h and showed the same pattern of Al accumulation.

When this experiment was repeated with 3 μmol Al·L^{-1} plus Suwannee River NOM at 5 mg C·L^{-1}, there was no accumulation of Al by trout gills at any pH (Fig. 1*a*) (not significantly different ($P = 0.975$) from control values at pH 7). Similar to the first experiment, the trout at pH 4.1 and 9.8 did not survive for 24 h, but now, trout at pH 8.0 and 8.8 also showed poor survival (Table 2). Gill Al concentrations for surviving trout sampled at 48 h are also given (Table 2);

Fig. 1. (*a*) Gill aluminum (Al) concentrations for rainbow trout (*Oncorhynchus mykiss*) exposed to 3 μmol Al·L^{-1} alone (solid bars) and to Al plus Suwannee River natural organic matter (NOM) at 5 mg C·L^{-1} (hatched bars) over a water pH range of pH 4–10. Bars represent gill Al concentrations after 3-h exposures (mean ± 1 SE, $n = 5$). Asterisks represent significant differences between gill Al values with and without NOM at a given pH (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). Water pH near the gills (i.e., in the gill microenvironment, in parentheses) were estimated from Fig. 1*c*. (*b*) Measured water Al concentrations for 0.45-μm-filtered water samples (i.e., dissolved Al) taken at 1 h from each exposure to 3 μmol Al·L^{-1} without (solid bars) and with (hatched bars) NOM at 5 mg C·L^{-1}. (*c*) Differences between expired water pH and inspired water pH as poorly buffered, ion-poor water passes over the gills of rainbow trout, modified from Fig. 1 of Playle and Wood (1989*a*). NH_3 released at the gills renders acidic water more basic as it passes over the gills (positive pH difference), and basic water is made more acidic by carbon dioxide released at the gills (negative pH difference).

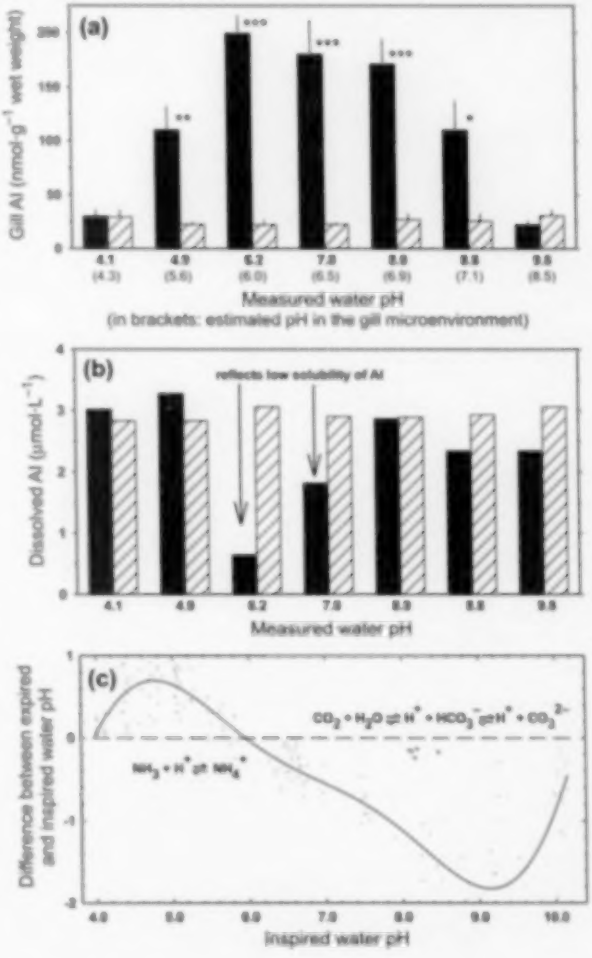

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Table 1. Water chemistry and rainbow trout (*Oncorhynchus mykiss*) gill aluminum (Al) for exposures to 3 μmol Al·L^{-1} alone at water pH 4–10.

Treatment	Measured pH	Sample time (h)	Gill Al (nmol·g wet weight^{-1})	Time to 50% mortality	Water Al (μmol·L^{-1})		TOC (mg C·L^{-1})	DOC (mg C·L^{-1})	Ca (μmol·L^{-1})	Na (μmol·L^{-1})	Cl (μmol·L^{-1})
					Total	Dissolved					
pH 4	4.1	1 or 3	28.8±6.8 (5)	22 h (100% mortality)	3.10	3.01	0.4	0.5	5.3	853	151
	4.0	24	—		2.70	3.05	0.6	0.7	6.3	847	121
	—	48	—		—	—	—	—	—	—	—
pH 5	5.0	1 or 3	109.8±22.9 (5)	>48 h (20% mortality)	3.18	3.27	0.4	1.8	6.4	860	109
	4.9	24	—		3.03	3.27	0.8	0.7	8.4	873	103
	4.8	48	165.8±22.0 (4)		3.09	2.94	0.4	0.5	7.0	891	121
pH 6	6.2	1 or 3	199.2±16.6 (5)	>48 h (20% mortality)	2.68	0.64	0.5	0.7	6.6	852	115
	6.2	24	—		0.96	0.62	0.9	0.6	4.3	891	145
	6.0	48	373.5±166.9 (4)[a]		0.88	0.52	0.5	0.6	7.8	869	133
pH 7	7.0	1 or 3	180.0±31.3 (5)	>48 h (0% mortality)	2.88	1.81	0.7	0.5	8.4	837	121
	7.2	24	—		2.21	1.48	0.7	0.6	6.2	856	115
	7.2	48	206.5±27.3 (5)		1.79	0.88	0.6	0.6	4.1	866	139
pH 8	8.0	1 or 3	171.0±23.5 (5)	>48 h (0% mortality)	3.06	2.86	0.5	0.5	3.9	879	121
	7.9	24	—		2.89	2.08	1.0	1.0	3.6	873	121
	7.9	48	236.9±15.9 (5)		2.71	2.71	0.5	0.6	6.8	903	133
pH 9	8.9	1 or 3	109.7±27.8 (5)	>48 h (20% mortality)	3.14	2.34	0.5	0.4	6.7	878	139
	8.7	24	—		2.62	2.88	0.7	0.6	5.1	884	169
	8.6	48	130.8±27.3 (4)		3.07	2.88	2.1	0.7	8.7	887	151
pH 10	9.9	1 or 3	22.0±4.0 (5)	5 h (100% mortality)	3.08	2.34	0.6	0.5	3.5	883	121
	—	24	—		—	—	—	—	—	—	—
	—	48	—		—	—	—	—	—	—	—
Control	7.2	1 or 3	28.2±4.6 (5)	>48 h (20% mortality)	0.03	0.05	0.4	0.4	5.9	852	115
	7.2	24	—		0.04	0.03	0.6	1.1	6.0	851	139
	7.2	48	62.2±38.8 (4)[a]		0.04	0.06	1.5	0.6	5.2	857	121

Note: Gills of trout were sampled for Al accumulation at 3 and 48 h (if available). Means ± 1 SE (*n*) are given. Time to 50% trout mortality is given as well as fish mortality at 48 h (in parentheses; e.g., 20% mortality = one of five trout dead). Water samples were taken at 1, 24, and 48 h. TOC, total organic carbon; DOC, dissolved organic carbon.

[a]One high value.

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Table 2. Water chemistry and rainbow trout (*Oncorhynchus mykiss*) gill aluminum (Al) for exposures to 3 μmol Al·L^{-1} plus Suwannee River natural organic matter (NOM) at 5 mg C·L^{-1} at water pH 4–10.

					Water Al (μmol·L^{-1})						
Treatment	Measured pH	Sample time (h)	Gill Al (nmol·g wet weight^{-1})	Time to 50% mortality	Total	Dissolved	TOC (mg C·L^{-1})	DOC (mg C·L^{-1})	Ca (μmol·L^{-1})	Na (μmol·L^{-1})	Cl (μmol·L^{-1})
pH 4	3.8	1 or 3	29.4±6.8 (5)	12 h (100% mortality)	2.91	2.83	4.1	4.3	6.0	815	164
	—	24	—		—	—	—	—	—	—	—
	—	48	—		—	—	—	—	—	—	—
pH 5	4.9	1 or 3	22.6±2.1 (5)	>48 h (20% mortality)	2.99	2.83	7.2	4.7	7.2	900	164
	5.0	24	—		3.12	2.71	4.7	4.7	8.0	886	164
	5.1	48	65.6±21.4 (4)[a]		3.00	2.87	5.4	4.8	7.2	894	188
pH 6	6.2	1 or 3	22.1±4.2 (5)	>48 h (20% mortality)	3.24	3.06	4.3	4.3	7.1	839	152
	6.1	24	—		2.96	2.91	4.8	9.1	6.7	873	158
	6.0	48	21.7±8.1 (4)		2.96	2.87	5.2	4.9	6.8	834	164
pH 7	7.0	1 or 3	22.6±1.9 (5)	>48 h (20% mortality)	2.95	2.90	4.5	4.4	7.6	854	158
	7.2	24	—		2.85	2.93	4.7	4.7	6.0	844	164
	7.1	48	25.4±9.9 (4)		3.03	2.80	5.6	4.9	7.7	840	170
pH 8	7.8	1 or 3	27.2±5.6 (5)	25 h (100% mortality)	3.01	2.89	4.4	4.4	7.5	861	164
	7.8	24	—		2.77	2.76	4.7	4.6	7.1	873	170
	—	48	—		—	—	—	—	—	—	—
pH 9	8.8	1 or 3	27.9±6.3 (5)	26 h (100% mortality)	3.05	2.93	4.4	4.4	6.6	858	182
	8.4	24	—		3.03	—[b]	4.9	4.9	7.1	873	207
	—	48			—	—	—	—	—	—	—
pH 10	9.7	1 or 3	30.6±5.8 (5)	12 h (100% mortality)	2.96	3.06	4.3	4.4	7.4	860	170
	—	24	—		—	—	—	—	—	—	—
	—	48	—		—	—	—	—	—	—	—
Control	7.0	1 or 3	26.9±4.8 (5)	>48 h (20% mortality)	0.22	—	4.4	—	5.6	809	170
	7.3	24	—		0.23	—	4.5	—	7.7	843	182
	7.2	48	23.8±8.1 (4)		0.21	—	5.3	—	6.0	818	195

Note: Gills of trout were sampled at 3 h and 48 h (if available). Means ± 1 SE (*n*) are given. Time to 50% mortality is given as well as fish mortality at 48 h (in parentheses; e.g., 20% mortality = one of five trout dead). Water samples were taken at 1, 24, and 48 h. TOC, total organic carbon; DOC, dissolved organic carbon.

[a]One high value.

[b]Lost sample.

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these values were significantly lower than those for the Al-alone exposures and at pH 7 were not significantly greater than control values.

Without NOM, less Al in the pH 6.2 and 7.0 waters passed through 0.45-μm filters, whereas the addition of NOM kept Al in solution at these water pHs (Fig. 1*b*). These total and dissolved Al concentrations demonstrate the low solubility of Al at pH 6 and 7 without NOM, and the 24- and 48-h Al data (Table 1) indicate progressive loss of Al from solution throughout the experiment at pH 6 and 7 (e.g., dissolved Al less than total Al). In contrast, Suwannee River NOM kept Al in solution for 48 h at pH 6 and 7 (Table 2).

Acute toxicity of pH 10 water with Al, NOM, and Ca

The addition of Suwannee River NOM at 5 mg C·L^{-1} to pH 10, ion-poor water resulted in 100% trout mortality in just 3 h (Fig. 2). The addition of 3 μmol Al·L^{-1} as well as the NOM increased the time to 100% trout mortality to 5 h. In pH 10 water with or without Al, the time to 100% mortality was 7 h. Addition of 450 μmol Ca·L^{-1} to the pH 10 water with NOM eliminated trout mortality to that of the control treatment at neutral pH. Water chemistry in these exposures was essentially the same as for comparable exposures (Tables 1 and 2) with about 450 μmol Ca·L^{-1} and 950 μmol Cl·L^{-1} in the high-Ca treatment.

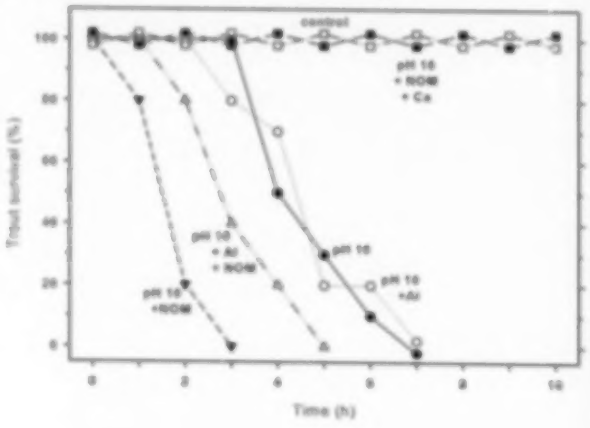
Discussion

Aluminum alone

Aluminum at pH 4 and 10 did not accumulate more than in trace amounts at the gills of rainbow trout, apparently because Al is highly soluble at both of these extreme water pHs plus ionic competition at the gills. The low pH effect on gill Al accumulation is the same as found by Playle and Wood (1989*b*), where high concentrations of H^+ also likely competed against Al^{3+} in solution from binding to the gill surface and caused themselves increased fish mortality. Elevated H^+ (low pH) in combination with high Al and low Ca concentrations can adversely affect critical life stages of fish (reviewed by Gensemer and Playle 1999; also see Keinänen et al. 1998). Thus, in very acidic conditions, the effects of Al may be overshadowed by the direct effects of low water pH, as has been documented by others (e.g., Muniz and Leivestad 1980), and instead of respiratory effects caused by accumulation of Al on the gills, ionoregulatory dysfunction is a prevailing toxic mechanism at very acidic pH (Neville 1985; reviewed by Gensemer and Playle 1999).

At pH 10, the predominate species of Al is the aluminate anion $Al(OH)_4^-$. In addition to the high solubility of Al at high pH, fish gills are generally thought to be negatively charged, so $Al(OH)_4^-$ would not be attracted to the gill surface and the fish would therefore not accumulate Al on the gills (Gensemer and Playle 1999; Poléo and Hytterød 2003). Although a 3-week exposure to 13 μmol Al·L^{-1} at pH 9.5 caused some physiological stress to Atlantic salmon (*Salmo salar*), Poléo and Hytterød (2003) concluded that the interaction of $Al(OH)_4^-$ at the gill surface and therefore its toxicity is very low. In another study done at pH 8.7, the addition of 19 and 37 μmol Al·L^{-1} (added as alum, $Al_2(SO_4)_3$) actually increased the survival of cannulated rainbow trout (from 43% at pH 8.7 alone to 75% and 100% survival over 96 h,

Fig. 2. Percent survival of 10 rainbow trout (*Oncorhynchus mykiss*) exposed for 10 h to low Ca, pH 10 water with the addition of Suwannee River natural organic matter (NOM) at 5 mg C·L^{-1}, 3 μmol Al·L^{-1}, NOM plus Al, and NOM plus 450 μmol Ca·L^{-1}. Symbols have been offset for clarity where necessary. Control exposures were 6 μmol Ca·L^{-1} at pH 7.



respectively; Heming and Blumhagen 1988); 370 μmol Al·L^{-1} was equally toxic to pH 8.7 alone, and 3700 μmol Al·L^{-1} was more toxic than pH 8.7 alone. Disruption of plasma Na and Cl concentrations was the toxic effect of base conditions alone, and the generally ameliorative effects of Al at pH 8.7 were speculated to have been due to $Al(OH)_4^-$ competition with OH^- at binding sites on the gills (Heming and Blumhagen 1988).

Most Al accumulated on the gills of rainbow trout at intermediate water pH because near pH 6, most Al occurs as polymers (Exley et al. 1996; Poléo 1995) or as insoluble amorphous $Al(OH)_3$, which then forms gibbsite (reviewed by Gensemer and Playle 1999); these reaction kinetics are rapid (e.g., Broshears et al. 1996) and were reflected in our dissolved Al measurements. Aluminum polymers or amorphous $Al(OH)_3$ accumulates on the gill surface of fish and can be toxic through respiratory disturbances. Because of Al polymerization and precipitation, it was expected that trout gills would accumulate Al over the pH 6–8 range, but some accumulation of Al was also observed at pH 4.9 and 8.8 where the high solubility of Al and competition by H^+ and the predominance of $AlOH_4^-$, respectively, would be expected to minimize Al binding to the gills.

Water chemistry in the gill microenvironment can also help explain Al accumulation by trout gills at pH 4.9 and 8.8. Water pH in the gill microenvironment was estimated from measured water pH plus previous data (Playle and Wood 1989*a*, 1989*b*). It is reasonable to apply the previous data from adult rainbow trout to our results using small rainbow trout because similar data were generated for small fathead minnows (Playle et al. 1992), indicating that these pH changes in the gill microenvironment are a general phenomenon of fish in ion-poor water. For our Al exposures at pH 4.9–8.8, estimated water pH in the gill microenvironment ranged from pH 5.6 to 7.1. As a result, Al in the inspired water would polymerize and precipitate in the gill microenvironment onto the gill surface; Al precipitation and poly-

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merization are rapid enough to occur at fish gills (1–4 s in Playle and Wood 1990; Jugdaohsingh et al. 2001). The greatest accumulation of Al by trout gills occurred in the pH 6.2–8.0 exposures, where the pH of the gill microenvironment was estimated to be pH 6.0–6.9; these water pHs correspond to low dissolved Al concentrations (Al polymerization and precipitation highest) at bulk water pH 6.2 and 7.0. That is, when Al solubility was low owing to Al polymerization and precipitation, accumulation of Al by the gills was high, as has been shown in mixing zones (e.g., Poléo and Bjerkely 2000; Henry et al. 2001; Kroglund et al. 2001).

Aluminum plus NOM

Addition of Suwannee River NOM eliminated Al accumulation on trout gills. Based on water sample measurements, it is clear that the added NOM complexed Al, keeping it in solution, but that the complexed Al was unavailable to interact with the gills even at water pH 6.2 and 7.0. The NOM used for this experiment was concentrated from a natural, allochthonous (terriginous) source, which is optically dark, indicating many aromatic groups (e.g., from lignin). This type of NOM tends to bind metals like Cu and Pb more strongly than optically lighter, autochthonous-like NOM (De Schamphelaere et al. 2004; Luider et al. 2004; Schwartz et al. 2004); we have some preliminary data suggesting that the same relationship exists for Al (A.R. Winter and R.C. Playle, unpublished data).

Protective effects of NOM against Al toxicity have been demonstrated for quite some time (e.g., Neville 1985; Witters et al. 1990; other studies reviewed in Gensemer and Playle 1999). More recently, Peuranen et al. (2003) showed that humic acid at 15 mg C·L^{-1} decreased Al deposition on the gills of grayling (*Thymallus thymallus*), decreased gill damage, and decreased the physiological effects of 3.7 µmol Al·L^{-1} at pH 5.5. In weakly alkaline water (pH ~ 8.2), Gundersen et al. (1994) found that humic acid at 10 mg C·L^{-1} decreased the 96-h acute toxicity of up to 185 µmol Al·L^{-1} (~18 µmol dissolved Al·L^{-1}); humic acid at 5 mg C·L^{-1} essentially eliminated toxicity of 19–74 µmol total Al·L^{-1} over 16 days but not the toxicity of 350 µmol total Al·L^{-1} (~44 µmol dissolved Al·L^{-1}), a very high concentration of Al. Fulvic acid protected fish from the toxic effects of Al but preexposure of fish to fulvic acid did not, and the protection by fulvic acid did not persist when the fish were transferred to an Al solution without fulvic acid (Roy and Campbell 1997). Thus, it is Al complexation by NOM that decreases the toxic effects of Al, not some other property of the NOM.

pH 10 toxicity

Juvenile rainbow trout did not accumulate Al on their gills at pH 10, but this exposure was very toxic to the fish on its own, particularly in our very ion-poor water. Alkaline conditions (pH > 9) caused gill damage, decreased plasma ion concentrations, and decreased NH_3 elimination in fish, which can result in fish mortality (e.g., Wilkie and Wood 1991, 1994; Lease et al. 2003). Poléo and Hytterød (2003) did not report mortality of Atlantic salmon at pH 9.5 (with or without Al) and suggested a narrow limit for the survival of salmonids (e.g., acute toxicity between pH 9.5 and 10.0). In our exposures, the addition of NOM increased the acute toxicity of pH 10 water, while Al alone was neither detrimental nor beneficial to the fish at pH 10. Aluminum decreased the adverse effects of NOM at pH 10.

From these results, it appears that in ion-poor pH 10 water, NOM can tie up the limited amount of Ca, interfering with ion homeostasis, and cause faster fish mortality. In support of this, the addition of 450 µmol Ca·L^{-1} to the pH 10 plus NOM treatment eliminated fish mortality, and the intermediate toxicity of pH 10 water with NOM plus Al suggests that Al binding to NOM decreased the number of sites on NOM otherwise available to bind Ca. This tying up of Ca by NOM is an indirect effect of NOM (Steinberg 2003), which we have seen occasionally in our laboratory with other metals in ion-poor water (e.g., Pb; Schwartz et al. 2004). This effect at pH 10 is the clearest demonstration of an indirect effect of NOM seen to date by us and, conversely, illustrates the general effect of Ca amelioration of acutely toxic agents. Another nice illustration of this type of indirect effect of a complexing agent was by Chowdhury and Blust (2002), where Sr uptake by gills of common carp (*Cyprinus carpio*) increased in the presence of the complexing agents nitrilotriacetate and ethylenediaminetetraacetic acid owing to decreased Ca competition at the gills because these two synthetic complexing agents have a 10- to 100-fold higher affinity for Ca^{2+} than for Sr^{2+}.

In summary, when juvenile rainbow trout were exposed to Al at pH 4–10 in ion-poor water, Al was accumulated by their gills at pH 5–9. Competition by H^+ in very acidic conditions plus the high solubility of Al decreased Al^{3+} binding to the negatively charged gills, whereas in very basic conditions, the aluminate anion $Al(OH)_4^-$ did not bind to the negatively charged gills. Aluminum accumulation by trout gills at more moderate conditions is a result of Al polymerization and precipitation phenomena and may be explained better when changes in water pH in the gill microenvironment are considered. Even though NOM kept more Al in solution at intermediate water pH, the Al was rendered unavailable to bind to the gills. These results suggest that Al–gill binding models and biotic ligand models can be created considering positively charged inorganic Al species (e.g., Al^{3+}) interacting at fish gills, Al polymerization and precipitation at the gills, and Al complexation by NOM. Water at pH 10 itself was acutely toxic to rainbow trout, and survival time decreased with the addition of NOM. Elimination of the acute lethal toxicity at pH 10 by adding Ca indicated that the adverse effect of organic matter was indirect through complexation of the limited amount of Ca in the ion-poor water.

Acknowledgements

We thank two anonymous reviewers for their constructive comments on the manuscript. This research was funded by the Natural Sciences and Engineering Research Council of Canada, which includes financial support from Noranda Inc. – Falconbridge Ltd., BHP Billiton Base Plc, and Wilfrid Laurier University.

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Reach-scale effects of riparian forest cover on urban stream ecosystems

Allison H. Roy, Christina L. Faust, Mary C. Freeman, and Judith L. Meyer

Abstract: We compared habitat and biota between paired open and forested reaches within five small streams (basin area 10–20 km^2) in suburban catchments (9%–49% urban land cover) in the Piedmont of Georgia, USA. Stream reaches with open canopies were narrower than forested reaches (4.1 versus 5.0 m, respectively). There were no differences in habitat diversity (variation in velocity, depth, or bed particle size) between open and forested reaches. However, absence of local forest cover corresponded to decreased large wood and increased algal chlorophyll *a* standing crop biomass. These differences in basal food resources translated into higher densities of fishes in open (9.0 individuals·m^{-2}) versus forested (4.9 individuals·m^{-2}) reaches, primarily attributed to higher densities of the herbivore *Campostoma oligolepis*. Densities of terrestrial invertebrate inputs were higher in open reaches; however, trends suggested higher biomass of terrestrial inputs in forested reaches and a corresponding higher density of terrestrial prey consumed by water column feeding fishes. Reach-scale biotic integrity (macroinvertebrates, salamanders, and fishes) was largely unaffected by differences in canopy cover. In urbanizing areas where catchment land cover drives habitat and biotic quality, management practices that rely exclusively on forested riparian areas for stream protection are unlikely to be effective at maintaining ecosystem integrity.

Résumé : Nous avons comparé les habitats et les organismes vivants dans des sections appariées, boisées et ouvertes, dans cinq petits ruisseaux (surface du bassin 10–20 km^2) dans des bassins versants de banlieue (9–49 % d'utilisation urbaine des terres) dans le piémont de Géorgie, É.-U. Les sections ouvertes de ruisseau sont plus étroites que les sections boisées (4,1 m vs 5,0 m, respectivement). Il n'y a pas de différence de diversité des habitats (variation de vitesse de courant, profondeur, granulométrie du lit) entre les sections ouvertes et boisées. Cependant, l'absence de couverture forestière locale entraîne une diminution de la quantité de bois de grande taille et une augmentation de la biomasse de chlorophylle *a* des algues. Ces différences de ressources alimentaires de base amènent des densités plus élevées de poissons dans les sections ouvertes (9,0 individuels·m^{-2}) que dans les sections boisées (4,9 individuels·m^{-2}), ce qui s'explique par des densités plus grandes de l'herbivore *Campostoma oligolepis*. La densité des apports d'invertébrés terrestres est plus grande dans les sections ouvertes; par contre, les tendances indiquent une biomasse plus grande des apports terrestres dans les sections boisées et une densité correspondante plus élevée de proies terrestres consommées par les poissons qui se nourrissent dans la colonne d'eau. L'intégrité biotique (macroinvertébrés, salamandres, poissons) au niveau des sections est généralement peu affectée par la couverture végétale. Dans les zones urbanisées où la couverture du sol du bassin versant détermine la qualité de l'habitat et de la communauté, les méthodes de gestion qui se basent exclusivement sur les zones riparienes boisées comme moyen de protection des cours d'eau risquent de ne pas réussir à maintenir l'intégrité de l'écosystème.

[Traduit par la Rédaction]

Introduction

Over the past two decades, research has highlighted the importance of riparian corridors as critical areas of the landscape for influencing stream ecosystem processes and stream biotic integrity (Correll 2000). These streamside areas have been labeled buffers because of their capacity to take up or retain nutrients (Peterjohn and Correll 1984; Osborne and Kovacic 1993), sediments (Davies and Nelson 1994; Waters 1995), metals, and other contaminants (also see reviews in Gregory et al. 1991; Sweeney 1992; Lowrance 1998). Forested riparian corridors also provide bank and geomorphic stability, large woody debris, and other detrital inputs (Trotter 1990) and terrestrial arthropod inputs (Mason and Mac-

Received 1 October 2004. Accepted 26 May 2005. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on 23 September 2005.
J18334

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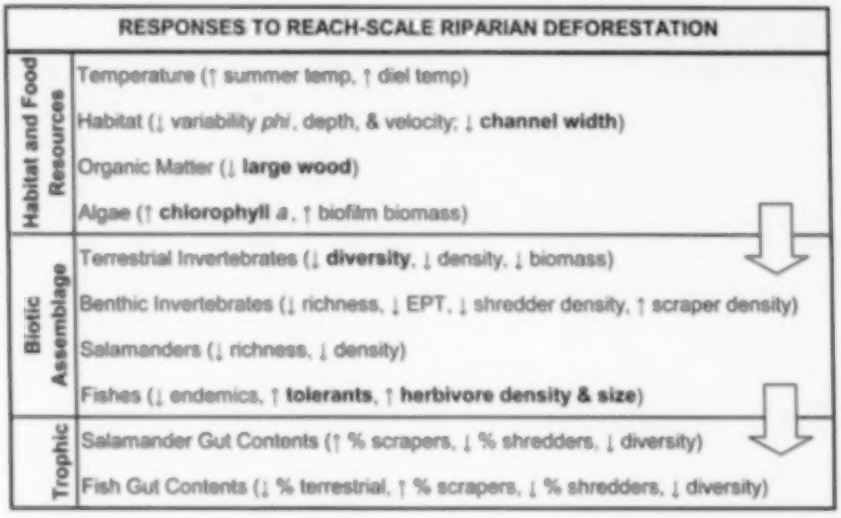
 doi: 10.1139/F05-135 © 2005 NRC Canada

Fig. 1. Conceptual model of relationships between reach-scale riparian deforestation and responses of basal food resources and consumers in urban streams. Riparian deforestation is predicted to result in changes in habitat (e.g., temperature and habitat diversity) and food resources (e.g., organic matter inputs and algae). We predict corresponding changes in biotic assemblages, including terrestrial invertebrates, benthic invertebrates, salamanders, and fishes. For example, reductions in terrestrial-derived carbon and increases in autochthonous carbon are predicted to increase densities of algae-eating invertebrates and fishes and decrease densities of shredding insects. We hypothesize that these changes in basal food resources will also be translated into differences in the proportions of prey types consumed by salamanders and fishes between open and forested reaches. Responses in bold indicate significant effects observed in this study.


RESPONSES TO REACH-SCALE RIPARIAN DEFORESTATION
Habitat and Food Resources
Temperature (↑ summer temp, ↑ diel temp)
Habitat (↓ variability *phi*, depth, & velocity; ↓ **channel width**)
Organic Matter (↓ **large wood**)
Algae (↑ **chlorophyll *a***, ↑ biofilm biomass)
Biotic Assemblage
Terrestrial Invertebrates (↓ **diversity**, ↓ density, ↓ biomass)
Benthic Invertebrates (↓ richness, ↓ EPT, ↓ shredder density, ↑ scraper density)
Salamanders (↓ richness, ↓ density)
Fishes (↓ endemics, ↑ **tolerants**, ↑ **herbivore density & size**)
Trophic
Salamander Gut Contents (↑ % scrapers, ↓ % shredders, ↓ diversity)
Fish Gut Contents (↓ % terrestrial, ↑ % scrapers, ↓ % shredders, ↓ diversity)

Donald 1982; Kawaguchi and Nakano 2001). For streams in forested landscapes, removal of riparian forests decreases shading, increases temperature (Beschta and Taylor 1988), and increases primary productivity (Murphy et al. 1981; Noel et al. 1986), which influences the biotic communities by favoring certain species and altering population dynamics (Barton et al. 1985; Feminella et al. 1989). Many studies have demonstrated how structural habitat provided by riparian buffers indirectly affects higher trophic levels such as macroinvertebrates (Benke et al. 1988; Whiles et al. 2000) and fishes (Karr and Schlosser 1978; Jones et al. 1999). Essentially, forested riparian areas supply streams with a high diversity and quality of instream habitat for aquatic biota.

Although intact riparian corridors have been shown to minimize degradation of water quality and biotic integrity resulting from catchment land use disturbance, this research has primarily been conducted in watersheds with agricultural (e.g., Peterjohn and Correll 1984; Osborne and Kovacic 1993) and silvicultural (e.g., Beschta and Taylor 1988; Davies and Nelson 1994) land uses. Very little is known about the function of riparian forests in urban and suburban areas (but see Hession et al. 2003; Sweeney et al. 2004). We anticipate that riparian forests may not be sufficient to protect streams in urban settings because (*i*) these corridors are often bypassed by stormwater conveyances and (*ii*) the instream impacts of urban land cover in the catchment (e.g., owing to hydrologic alteration and increased pollutant delivery) are overwhelming. However, at the reach scale, forested riparian areas may still provide essential functions of shading, bank stability, and organic matter inputs for streams.

This study directly addressed the influence of reach-scale (200 m) riparian forests on habitat and biota in urbanizing streams. We compared water quality, physical habitat, food resources (e.g., algae), benthic and terrestrial invertebrates, salamanders, and fishes between paired open and forested reaches within five urban streams. We illustrated the predicted changes associated with reach-scale riparian deforestation (Fig. 1). Although riparian forests have been shown to reduce nutrient and sediments inputs to streams, we did not expect differences in water quality between adjacent open and forested reaches because of the close proximity of the reaches. Stream reaches without forested canopies are hypothesized to have higher mean annual temperature and temperature variability (Beschta and Taylor 1988; Sweeney 1992), reduced habitat diversity (e.g., variation in depth, velocity, and bed sediment size; Jones et al. 1999), reduced channel width (Davies-Colley 1997; Scarsbrook and Halliday 1999; Sweeney et al. 2004), reduced organic matter (Sweeney 1992), and increased algae (McIntyre and Phinney 1965). We also hypothesize that streams with lower canopy cover will have lower diversity, density, and biomass of terrestrial invertebrate inputs (Mason and MacDonald 1982; Edwards and Huryn 1996; Kawaguchi and Nakano 2001). Differences in habitat quality are expected to translate into differences in assemblage integrity of benthic macroinvertebrates, salamanders, and fishes. Shifts from allochthonous to autochthonous food resources with riparian deforestation are expected to result in decreased densities of shredding invertebrates dependent on leaf material (Wallace et al. 1999) and increased densities of herbivorous invertebrates (e.g., scrapers) and fishes. The relative changes in benthic and terrestrial invertebrates

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Table 1. Site characteristics for study streams within the Etowah River basin, Georgia.

	Clark	Copper Sandy	Lawrence	Picketts Mill	Westbrook
Location					
UTM Northing (NAD 1983)	717 590	746 302	701 124	708 236	707 590
UTM Easting (NAD 1983)	3 773 997	3 777 695	3 758 149	3 761 223	3 770 001
County	Cobb/Cherokee	Fulton	Paulding	Cobb/Paulding	Paulding/Cobb
Open reach					
Type of land use	Golf course	Horse/cow pasture	Ball fields	Horse pasture	Golf course
Location (relative to forest reach)	Upstream	Downstream	Downstream	Upstream	Downstream
Total length open (m)	554	350	348	368	400
Canopy cover (July 2002)					
Open reach (%)	10.6	44.2	62.8	23.0	20.8
Forest reach (%)	91.3	82.1	96.1	83.8	58.9
Basin and stream morphometry					
Drainage area (km^2)	16.2	16.0	11.8	15.1	18.9
Stream length (km)	36.9	34.2	24.4	34.1	46.2
Drainage density ($km \cdot km^{-2}$)	2.3	2.1	2.1	2.3	2.4
Elevation (m)	860	980	880	900	740
Stream slope	0.0034	0.0054	0.0035	0.0039	0.0034
Catchment land cover					
Impervious (%)	17.3	14.5	9.7	13.2	8.8
Urban (%)	49.3	20.5	8.8	25.6	19.5
Forest (%)	40.5	55.1	73.9	61.0	69.3
Agriculture (%)	3.5	13.0	9.1	7.8	4.2
Open water (%)	0.7	1.9	1.1	0.4	0.7
Riparian land cover (network)					
Urban (%)	16.3	5.5	2.9	8.3	2.4
Forest (%)	68.9	76.1	86.6	84.4	87.5
Agriculture (%)	2.3	8.8	5.3	3.8	6.1
Open water (%)	3.2	8.1	4.3	2.8	3.6
Road crossings ($no. \cdot km\ stream^{-1}$)	0.51	0.26	0.61	0.47	0.19
Impoundments ($no. \cdot km\ stream^{-1}$)	0.46	0.55	0.29	0.53	0.28

Note: Catchment land cover is based on 2001 Landsat Thematic Mapper satellite imagery. Riparian land cover is based on 1999 orthorectified aerial photographs. Reclassification of land cover subsequent to site selection (2001 versus 1997 data) resulted in <15% urban land cover in Lawrence Creek.

are expected to translate into diet shifts of insectivorous salamanders and fishes. Finally, we expected differential use of open and forest patches by small and large fish owing habitat availability, food availability, and (or) biotic interactions. Specifically, we hypothesized that herbivorous fishes would be larger in open reaches, reflecting higher growth rates owing to algal availability (Fig. 1). We tested these predicted differences between open and forested reaches to determine the function of reach-scale riparian forests in urban streams.

Methods

Study sites

This study took place in five small streams within the Piedmont physiographic region of the Etowah River basin, a 4823-km^2 catchment in north-central Georgia, USA. The region was originally dominated by deciduous forests; however, human practices in the last century have transformed the landscape into a mosaic of secondary forest, agriculture, and urban uses. In the last two decades, suburban development spreading from metropolitan Atlanta (population >4 million) has led to concerns of habitat degradation and species loss within the watershed (Burkhead et al. 1997). Specifically, catchments of small streams (basin area 10–20 km^2, approximately second to third order) within the Etowah River basin exhibited an average 13% decrease in forest cover and 11% increase in urban land cover between 1973 and 1997 (Roy et al. 2003*a*). Although on average, 1997 forest land cover was 5% higher in 100-m riparian corridors relative to the entire catchment, riparian areas also exhibited a 10% loss of forest cover and an 8% increase in urban land cover over the 24-year period (Roy et al. 2003*a*).

Five streams (Clark, Copper Sandy, Lawrence, Picketts Mill, and Westbrook Creeks) with paired sites in open and forested reaches (for a total of 10 sites) were selected for the study (Table 1). Streams were selected to be of similar size (drainage area from 10 to 20 km^2) and similar slope (ranging from 0.34% to 0.54%). We selected streams in catchments that had >15% urban land cover (i.e., above the level at which effects of urban disturbance on biota should be detectable; Schueler 1994) and <30% agriculture land cover based on 1997 Landsat Thematic Mapper satellite imagery. Open reaches consisted of at least 200 m of stream that had an open canopy because of lack of forest on at least one side of the stream. Forested reaches had at least a 30-m width of forest on each side of the stream for the entire 200-m-long

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reach. Less than 1 km of stream separated paired open and forested reaches and no significant tributaries entered between the paired reaches that would drastically change drainage area or upstream land cover. These five streams were the only streams with paired open and forested reaches that fit the design criteria within the Etowah River basin.

Landscape assessment

Subsequent to site selection, we recalculated land cover for each subcatchment with 2001 Landsat Thematic Mapper satellite imagery (30-m pixels) because this was the most recent and applicable coverage available. We also determined land cover types within a 30-m-wide buffer (corresponding to typical lot widths) for the drainage network upstream of the sample site using 1999 orthorectified aerial photography (1-m resolution). Land cover was classified as urban (low and high density), forest (evergreen, deciduous, and mixed), agriculture (cultivated/exposed land, and cropland/grassland), open water, or other (e.g., clearcut and golf course). We determined percent impervious cover for each subcatchment from a classified data set created by the Georgia Land Use Trends Project (Natural Resources Spatial Analysis Laboratory, Institute of Ecology, University of Georgia, Athens, GA 30602, USA). Department of Transportation road data from 1999 were used to calculate the number of road crossings per stream length for each site. Air photographs from 1999 were also used to count the number of stream impoundments, which was divided by stream length for comparison across streams.

Percent canopy cover was measured at the stream centerline at 10 locations along each reach in July 2002 using a spherical densiometer. At 10 random transects (corresponding to terrestrial invertebrate input transects), we measured distance from stream bank and diameter at breast height for all trees within a 5-m-wide corridor extending 30 m from the stream bank. Tree density (number of trees per square metre), mean tree diameter, and basal area of trees per hectare were calculated from tree measurements.

Environmental variables

Stream water chemistry was sampled five times from 7 November 2001 to 31 August 2002. Dissolved oxygen was measured with a portable Hydrolab Datasonde™ 4 (Hydrolab Corporation, Austin, Texas), which was calibrated before each use. Turbidity was measured in the field with a Hach™ turbidimeter (HACH Company, Loveland, Colorado). Grab water samples (taken halfway between bed and water surfaces) were filtered (Gelman® glass fiber, 0.45 µm; Gelman Sciences, Ann Arbor, Michigan) into acid-washed bottles, placed on ice, and transported to the laboratory for NH_4-N, NO_2/NO_3-N, and soluble reactive P analysis using an Alpkem autoanalyzer (Alpkem Corporation, Wilsonville, Oregon) following standard methods protocol (American Public Health Association et al. 1989). We also calculated the molar ratio of dissolved N:P as dissolved inorganic N (NH_4-N + NO_2/NO_3-N) to soluble reactive P. Hobo™ temperature data loggers were installed in November 2001 and stream temperature was recorded every 15 min for 1 year.

Stream slope was measured at the water surface at tops of riffles with a TopCon™ survey scope and stadia rod for the 200-m reach. Percentages of riffle, pool, and run habitat were calculated based on visual designation of habitat units determined by professional judgment using depth, water surface, and bed texture. A visual estimate of percent fines and percent embeddedness was made in one random location within each habitat unit using a view box. Depth, velocity, and modal particle size (phi) were measured at 34 random distances (68 for centerline) in the 200-m reach along five longitudinal transects (10, 30, 50, 70, and 90 percentiles) in a zigzag manner for a total of 204 measurements. These variables were used to calculate the Shannon index (H') (Zar 1974) as a measure of habitat diversity for single variables and all combinations of depth, velocity, and particle size (following Jones et al. 1999). A measure of evenness (J') (relative diversity) was also calculated by dividing H' by the log of the number of variable categories (1–3; Zar 1974). A quantitative estimate of percent fines (<2 mm) in riffle habitats was obtained by taking three 1-L grab samples of the bed sediment and dry sieving and weighing the material in the laboratory.

Basal food resources

The amount of wetted large wood was measured for each 200-m sample reach. Diameter and length were measured for all submerged wood ≥10 cm in diameter and ≥1 m long to calculate total volume of large wood.

Biofilm and algal chlorophyll *a* were sampled during September–October 2003 during leaf-out at 10 equally spaced transects along the 200-m reach. Three samples were taken from the dominant substrate at each transect using a 60-mL syringe modified into a Loeb sampler (rock substrates) or sediment core (sand substrates). Samples were brought back to the laboratory on ice where sand samples were sonicated for 1 min. All samples were stirred for ~1 min and subsamples of a known volume of slurry were filtered onto Whatman® GF/F filters (Whatman Inc., Clifton, New Jersey) for chlorophyll *a* and biofilm biomass. Biofilm biomass samples were dried, ashed, and weighed to determine biofilm ash-free dry mass. Chlorophyll *a* was extracted from frozen filters by first cutting and grinding in 90% acetone to break apart any blue-green cells (adapted from Wetzel and Likens 1991). Samples were decanted into a centrifuge tube and placed in a refrigerator for ~24 h. Samples were then centrifuged for ~5 min, subsampled (if necessary), and analyzed for chlorophyll *a* with a fluorometer (TD-700; Turner Designs, Sunnyvale, California).

Invertebrates

Benthic invertebrates were sampled in five replicate riffle habitats (one sample per riffle) at each of the 10 study reaches between 8 and 15 April 2002 prior to most insect emergence. Invertebrates were collected using a 0.09-m^2 surber sampler (500-µm mesh) and scrubbing rocks to a depth of 10 cm for 3 min. Samples were elutriated in the field, separated into small (0.5–1 mm) and large (>1 mm) size fractions, and preserved in 10% formalin. In the laboratory, the small fraction was subsampled to 100 invertebrates using a wheel sample splitter when necessary. All invertebrates in the large fraction and the subsampled small fraction were counted and identified to genus except for Chirono-

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midae (classified as predatory Tanypodinae or collector–gatherers) and noninsects (identified to order) using standard keys (Merritt and Cummins 1996). Richness (number of taxa), density (number of individuals per square metre), and other macroinvertebrate composition measures (Ephemeroptera, Plecoptera, and Trichoptera richness and functional feeding group densities) were calculated.

Terrestrial invertebrate inputs into the stream were sampled 8–16 July 2002. Invertebrates were collected by placing 10 floating clear containers (surface area = 0.344 m^2 each) filled with water and unscented soap (surfactant) at random transects along the stream reach (based on methods similar to those in Mason and MacDonald 1982). Invertebrates were removed after 24 h and preserved in formalin. In the laboratory, invertebrates were identified to order, counted, and dried to determine biomass. Diversity was calculated using the Shannon–Weiner diversity index H'.

Salamanders

Stream reaches were surveyed for stream salamanders at the three streams with riffle habitat in both open and forested reaches during 30–31 March 2003. We used a backpack electrofisher (model 12-B; Smith-Root®, Inc., Vancouver, Washington) in leaf packs and shallow riffle areas while kicking rocks and organic debris and holding a 500-µm-mesh kick net downstream. We searched for equal amounts of time in open and forested reaches within each stream and recorded shock time. Salamanders were euthanized using an overdose of tricaine methanesulfonate (MS 222), preserved in 10% formalin, and brought back to the laboratory for identification and gut analysis. Area (square metres) of riffle habitat sampled for salamanders was measured to estimate salamander densities.

In the laboratory, salamanders were transferred to 70% ethanol. Each individual was counted and measured for snout–vent length. Salamander stomach contents were analyzed for all individuals in Lawrence Creek (four total) and Picketts Mill Creek (27 total) and a randomly selected subset of 12 individuals within each reach at Clark Creek. We removed the stomachs and intestines of individuals under a dissecting microscope (15× magnification). Gut contents were identified to lowest possible taxonomic unit (typically order or family) and counted. Salamander abundance, catch per unit effort time, diversity of prey items (H'), and percent abundance of dominant food item were calculated for each reach.

Fishes

In fall 2001, fish assemblages were sampled using a backpack electrofisher in the most efficient manner for the habitat present: a downstream seine was used for riffles, dip nets were used for deep pools, and seine hauls were used for shallow homogenous runs. Block nets were used to separate habitat units (riffle, riffle–run, pool, and pool–run) and each segment was fished for three passes. We sampled a minimum of three replicate riffle or riffle–run and pool or pool–run habitats for a total stream length of up to 200 m. Fishes were identified in the field, measured, and released. Individual fish that could not be identified (including young of the year) were euthanized using MS 222, preserved in 8% formalin, and brought to the laboratory for identification.

Fish data were used to calculate richness and density of various composition metrics. We assessed fish assemblage integrity with measures of tolerant species, habitat generalists, and cosmopolitan species (indicative of low assemblage integrity) and highland endemic species (indicative of high assemblage integrity). Tolerant species were defined as widespread ubiquitous species found in over 80% of small streams in the Etowah based on previous research (Walters et al. 2003) and are expected to be tolerant of multiple stressors (Appendix A). Habitat generalists were defined as species that do not show preference for pool, pool–run, or riffle–run habitats and were classified based on Etnier and Starnes (1993) and Mettee et al. (1996). Cosmopolitan species were defined as those fishes native to at least 10 major drainages, whereas endemic species were primarily limited to the Coosa River drainage (Walters et al. 2003). We also examined the ratio of endemics to cosmopolitans (based on species richness and abundance), a metric that should detect homogenization of fish assemblages coincident with a loss of endemic species (Scott and Helfman 2001; Walters et al. 2003). We assessed standard length of individuals to compare differences in fish size (as reflective of growth or year class) between open and forested reaches for all individuals and with young of the year (YOY) excluded.

Individuals of two invertivore species (blackbanded darter (*Percina nigrofasciata*) and Alabama shiner (*Cyprinella callistia*)) and two trophic generalist species (redbreast sunfish (*Lepomis auritus*) and Alabama hog sucker (*Hypentelium etowanum*)) collected during sampling in fall 2001 were brought back to the laboratory for gut content analysis. Coosa shiner (*Notropis xaenocephalus*), an invertivore, was collected instead of *C. callistia* at one site where the latter species was not encountered. In the laboratory, all individuals were measured and weighed and the contents of the stomachs and intestines were removed from the fishes. We identified the gut contents of a maximum of 10 individuals; if more than 10 individuals were collected, we selected individuals most similar in size between open and forested reaches at a stream. Gut contents were identified to the lowest possible taxonomic unit (typically order or family) and counted. Percent abundance of food types (at the level of order) and percent terrestrial (versus aquatic) food items were compared between open and forested reaches.

Data analysis

This study was designed to compare reaches with different amounts of riparian cover, with all other land cover factors being equal. Thus, paired t tests were used to compare mean differences between open and forested reaches in environmental, basal food resource, algae, invertebrate, salamander, and fish variables. Fish lengths were compared using two-sample t tests (assuming unequal variances) for each species that had greater than four individuals in open and forested reaches. Because open and forested reaches exhibited a wide range in percent canopy cover, scatterplots and linear regressions (r^2) were also used to assess environmental and biotic parameters relative to percent canopy cover. All percentage data were transformed using arcsine square

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Table 2. Comparison of riparian cover, water quality, selected physical habitat variables, and algae and biofilm between open and forested reaches (paired *t* test for means, df = 4).

	Open		Forest			
	Mean	SD	Mean	SD	*t*	*p*
Riparian trees and canopy cover						
Canopy cover (%)	**32.3**	**21.0**	**77.6**	**12.5**	**4.73**	**0.005**
Tree density (no.·m^{-2})[a]	**0.038**	**0.037**	**0.179**	**0.055**	**4.53**	**0.010**
Tree diameter (cm)[a]	22.2	12.3	11.6	2.6	1.68	0.096
Basal area (m^2) per hectare[a]	**108**	**80**	**482**	**212**	**3.66**	**0.018**
Water quality						
Baseflow discharge (m^3·s^{-1})	0.037	0.008	0.035	0.007	0.69	0.264
Dissolved oxygen (mg·L^{-1})	**9.4**	**1.0**	**8.9**	**1.0**	**2.51**	**0.033**
Turbidity (NTU)	12.8	9.2	10.8	4.7	0.82	0.229
SRP (μg·L^{-1})	0.008	0.010	0.005	0.003	0.52	0.315
NH_4-N (μg·L^{-1})	0.005	0.007	0.003	0.004	0.92	0.206
NO_2/NO_3-N (μg·L^{-1})	**0.114**	**0.033**	**0.126**	**0.038**	**5.71**	**0.002**
DIN:SRP	92.1	158.9	18.3	11.8	0.91	0.215
Temperature						
Annual mean (°C)[a]	15.60	2.20	15.24	2.01	1.78	0.075
Annual daily diel (°C)[a]	4.34	0.98	3.55	0.70	1.43	0.113
Summer daily minimum (°C)[a,b]	**21.06**	**0.88**	**20.13**	**0.17**	**2.34**	**0.051**
Summer daily maximum (°C)[a,b]	27.13	1.85	25.86	1.35	1.15	0.166
Channel morphology						
Riffle area (%)	17.2	10.1	27.1	18.4	1.12	0.148
Width mean (m)	**4.1**	**1.2**	**5.0**	**1.1**	**2.12**	**0.051**
Depth mean (m)	0.22	0.08	0.25	0.12	0.46	0.334
Large wood						
Total no. of pieces	**10.0**	**5.1**	**31.8**	**17.6**	**2.92**	**0.022**
Total volume (m^3)	0.4	0.4	1.3	1.3	1.51	0.103
Biofilm and algae						
Biofilm AFDM (g·m^{-2})	32.9	9.7	100.9	150.6	1.04	0.179
Chlorophyll *a* (mg·m^{-2})	**32.7**	**16.9**	**13.7**	**11.3**	**2.17**	**0.048**

Note: NTU, nephelometric turbidity units; DIN, dissolved inorganic nitrogen; SRP, soluble reactive phosphorus; AFDM, ash-free dry mass. Bold indicates $p \leq 0.051$.

[a]df = 3.

[b]Open reach of Clark Creek missing for 1 April to 26 June, replaced with forest data (i.e., conservative extrapolation); Westbrook data ended 11 June.

root, and abundance data were transformed using $\log(x + 1)$ or $x^{0.25}$ where necessary to normalize skewed distributions.

Results

Differences in environmental variables and basal food resources

Canopy cover at forested reaches (77.6%) was about twice that at open reaches (32.3%) (Table 2). Land cover adjacent to open reaches included golf courses, pasture, and ball fields (Table 1). Catchments upstream from our sites contained from 8.8% to 49.3% urban land cover (based on reclassification subsequent to site selection). Impervious cover in the catchments (9.7%–17.3%) and density of road crossings (0.19–0.61 m stream length^{-1}) also reflected the urban influence on these streams (Table 1). Riparian vegetation differed between reaches, with higher densities of trees in forested reaches ($p = 0.010$), although trees in open reaches tended to be larger in diameter than those in forested reaches (result not significant, $p = 0.096$) (Table 2).

In general, water quality was similar between open and forested reaches (Table 2). One notable exception was dissolved oxygen, with higher daytime concentrations in open (9.4 mg·L^{-1}) versus forested reaches (8.9 mg·L^{-1}). Additionally, NO_2/NO_3-N concentrations were higher in forested reaches versus in open reaches. Differences in canopy cover between paired reaches also affected stream temperature, with higher summer daily minimum temperature and trends (although not significant) toward higher diel temperatures in open reaches (Table 2).

There were no significant differences in percent riffle area between open and forested reaches (Table 2). Although there were no differences in depth between reaches, streams were on average about 20% wider in forested (5.0 m) versus open reaches (4.1 m). We hypothesized that riparian deforestation would alter in-stream habitat diversity; however, we found no evidence of differences in diversity (H') or evenness (J') of depth, velocity, or bed sediment size or any combination of those variables ($p = 0.31$–0.50) (see Roy 2004). As expected, the number of large wood pieces was higher in forested reaches compared with open reaches, although there were no significant differences in volume of large wood (Table 2).

Higher light associated with open canopies was expected to result in higher amounts of benthic algae. Although there

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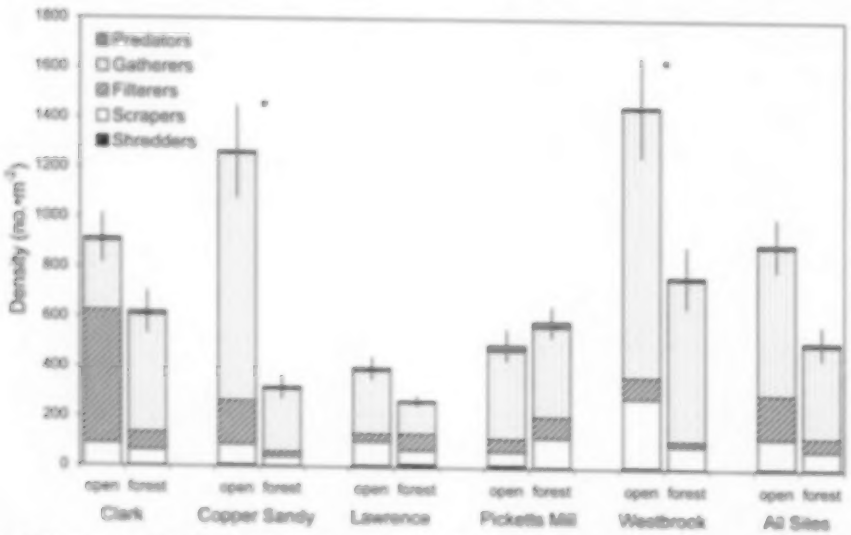
Table 3. Comparison of benthic invertebrate (mean of five replicates per site, df = 4) and total salamander (df = 2) measures between open and forested reaches (paired *t* test for means).

	Open		Forest			
	Mean	SD	Mean	SD	*t*	*p*
Benthic invertebrates						
Richness						
Total richness	16.8	10.6	6.1	8.1	1.01	0.185
Insect richness	13.1	8.1	5.4	6.0	1.06	0.075
Ephemeroptera, Plecoptera, and Trichoptera richness	5.5	3.6	2.5	1.9	0.38	0.361
Density						
Total density (no.·m^{-2})*	1131.5	822.3	633.8	535.4	1.75	0.077
SD total density (no.·m^{-2})	**586.8**	**447.1**	**378.4**	**292.2**	**2.74**	**0.026**
Scraper density (no.·m^{-2})*	120.0	88.3	72.9	31.6	1.31	0.131
Shredder density (no.·m^{-2})*	6.2	4.0	4.9	4.8	1.35	0.124
Salamanders (*Eurycea cirrigera*)						
Catch per unit effort	0.5	0.7	0.2	0.1	0.93	0.225
Snout–vent length (mm)	3.0	0.5	2.9	0.2	0.59	0.308
Stomach content density (*H'*)	1.2	0.8	1.3	0.5	0.64	0.294
Dominant food item abundance (%)	55.5	29.0	54.2	18.0	0.09	0.468

Note: Bold indicates $p < 0.05$.
*log(*x* + 1) transformed for analysis.

Fig. 2. Mean densities of benthic invertebrate functional feeding groups in open and forested reaches of each stream. Comparisons in overall mean densities between open and forested reaches within streams (two-sample *t* test assuming unequal variances, df = 4) and among all stream pairs ("all sites", paired *t* test for means, df = 4) are reported. Lines indicate ± standard errors for overall mean densities. *, $p < 0.05$.



was no significant difference in biofilm biomass between paired reaches (Table 2), biomass on rock substrates was marginally ($p = 0.051$) higher in open versus forested reaches (Roy 2004). We also found significantly higher concentrations of chlorophyll *a* in open versus forested reaches.

Differences in biotic assemblages

Benthic invertebrates in riffle habitats did not differ in total taxa richness or richness of insect or Ephemeroptera, Plecoptera, and Trichoptera taxa (Table 3). In addition, there were no significant differences in benthic invertebrate densities between paired reaches, although most streams had higher insect densities in open versus forested reaches (Fig. 2). The variation in densities among replicate samples (an indicator of patchiness) was significantly higher in open reaches compared with forested reaches. Scraper density (i.e., insects that primarily feed on benthic algae) was higher in open reaches compared with forested reaches in all sites except Picketts Mill Creek; however, the overall differences did not result in a significant effect of canopy cover. Shredders (i.e., insects that primarily feed on leaf material) accounted for a very small portion of the invertebrate density, and there were no differences in densities between reaches (Table 3; Fig. 2).

Terrestrial invertebrate inputs were numerically dominated by Diptera and Collembola at all sites (Fig. 3). We expected overall inputs to be higher in forested reaches, where insects

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Fig. 3. Mean densities of common terrestrial invertebrate groups (mean density >1 m^{-2}) in open and forested reaches of each stream. Comparisons in overall densities between open and forested reaches within streams (two-sample *t* test assuming unequal variances, df = 9) and among all stream pairs ("all sites", paired *t* test for means, df = 4) are reported. Lines indicate ± standard errors for overall mean densities. *, $p < 0.05$; **, $p < 0.01$.

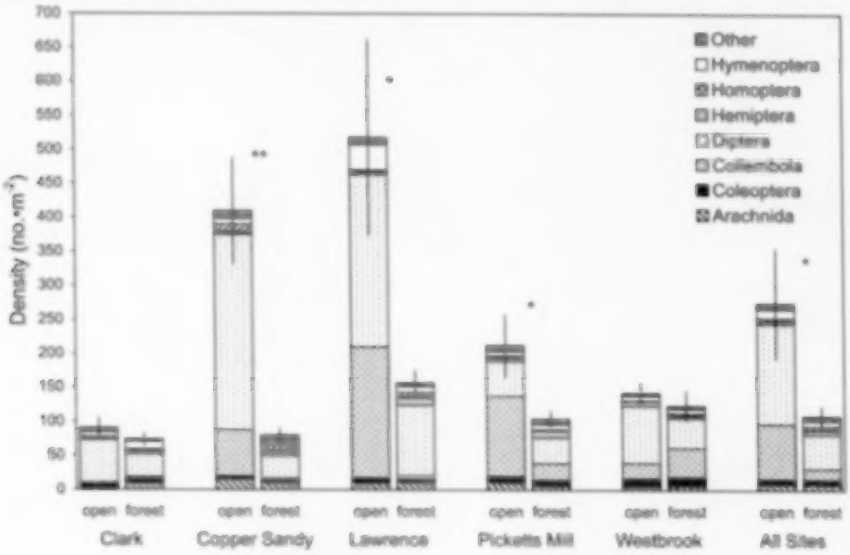

Table 4. Comparison between measures of fish assemblage composition and integrity between open and forested reaches (paired *t* test for means, df = 4).

	Open		Forest			
	Mean	SD	Mean	SD	*t*	*p*
Overall measures						
Species richness	16	2.9	14.8	3.0	1.63	0.089
Density (no.·m^{-2})	**9.0**	**2.4**	**4.9**	**1.7**	**4.78**	**0.004**
Relative abundance one dominant species	0.37	0.10	0.29	0.10	1.27	0.136
Endemic (E) and cosmopolitan (C) species						
Endemic richness	2	1	1.6	1.1	1.63	0.187
Endemic density (no.·m^{-2})	1.05	0.74	0.85	0.70	0.88	0.215
Cosmopolitan richness	**5.22**	**2.77**	**2.83**	**1.94**	**3.81**	**0.009**
Cosmopolitan density (no.·m^{-2})	8.6	1.1	8.2	1.8	1.00	0.187
E/(E + C) (abundance)[a]	0.16	0.11	0.24	0.21	1.64	0.088
Gut content diversity (H')						
Lepomis auritus	0.19	0.01	0.20	0.01	1.56	0.096
Cyprinella callistia	0.07	0.07	0.17	0.02	1.82	0.105
Percina nigrofasciata	0.11	0.01	0.11	0.01	0.37	0.370
Hypentelium etowanum	0.11	0.02	0.11	0.01	0.04	0.484

Note: Invertebrate prey diversity was assessed in four species of fish. Bold indicates $p < 0.05$.
[a]Abundance $x^{0.25}$ transformed prior to calculating the ratio of E to C or E to E + C.

were more likely to fall from the canopy into stream reaches. Contrary to our expectation, there was a higher density of terrestrial invertebrates in open (275 individuals·m^{-2}) versus forested reaches (108 individuals·m^{-2}) ($p = 0.048$), primarily driven by higher densities of Diptera in open reaches (Fig. 3). In contrast, forested reaches had higher mean terrestrial invertebrate biomass (0.092 g·m^{-2}) compared with open reaches (0.044 g·m^{-2}), although the difference was not significant ($p = 0.129$). Forested reaches also exhibited a higher diversity of terrestrial invertebrate inputs compared with open reaches ($H' = 1.68$ versus 1.28, $p = 0.019$).

The Southern two-lined salamander (*Eurycea cirrigera*) was the only salamander found at the three streams sampled, and all except three individuals were larvae. The catch per unit effort varied more across streams than between paired reaches, and there were no differences in salamander size between open and forested reaches (Table 3). Notably, at Clark Creek, a site with abundant cobble–gravel riffle habitat and highest salamander abundances, we captured 100 in-

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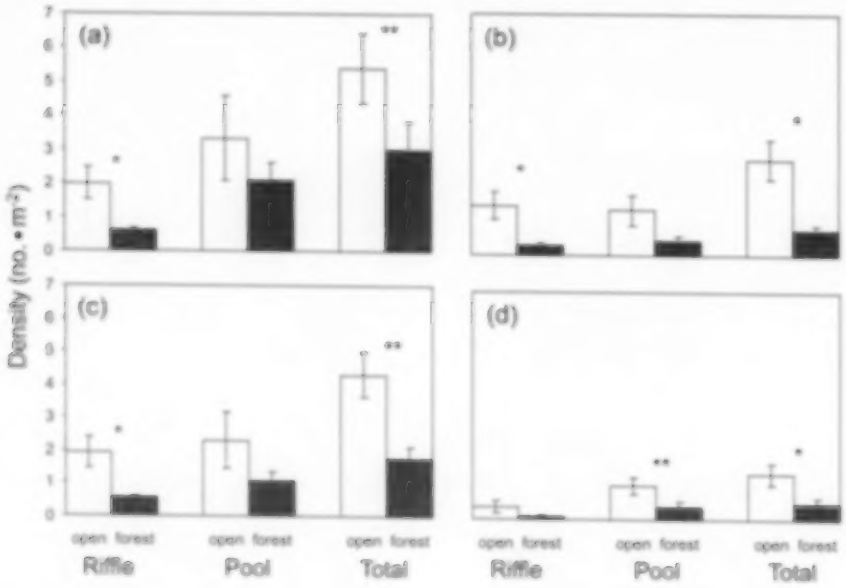
Fig. 4. Densities (mean ± 1 SE) of tolerant fish species groups and the two most abundant fish species in riffle habitats, pool habitats, and all habitats combined. Comparisons between mean densities for open (open bars) and forested (solid bars) reaches are reported (paired *t* test for means, df = 4). *, $p < 0.05$; **, $p < 0.01$. (*a*) Tolerants; (*b*) *Campostoma oligolepis*; (*c*) habitat generalists; (*d*) *Fundulus stellifer*.



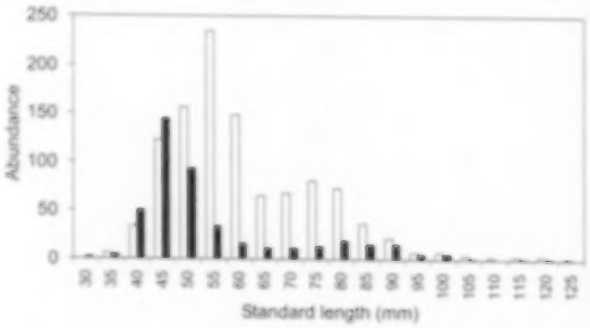
dividuals in the open reach compared with 18 in the forested reach. Larval salamander gut contents revealed similar diversity of diets and percent abundance of dominant food items between open and forested reaches (Table 3).

There were significantly higher densities of fishes in open versus forested reaches (Table 4). These higher overall densities in open reaches were driven by higher densities of the largescale stoneroller (*Campostoma oligolepis*), an herbivore, and the southern studfish (*Fundulus stellifer*) (Fig. 4). These comparisons were consistent across habitat type; riffle habitats had higher *C. oligolepis* densities in open reaches, pool habitats had higher *F. stellifer* densities in open reaches, and no other fish group showed significant differences between reaches within habitat types (Fig. 4).

There were no differences in fish assemblage integrity in terms of species richness or dominance by one species (Table 4). Trends suggested a higher abundance of endemics relative to cosmopolitan species in forested reaches (difference not significant, $p = 0.088$). There was significantly higher richness of cosmopolitan species (Table 4) and density of tolerant and habitat generalist species (Fig. 4) in the open reaches relative to the forested reaches. However, these differences were driven primarily by higher densities of *C. oligolepis*, which is considered a cosmopolitan, tolerant, and habitat generalist species (Appendix A).

Four cyprinid fish species were significantly larger in forested reaches, and for all species except *C. callistia*, these differences were driven by YOY cyprinids collected in the open reach of Westbrook Creek (Appendix A). Black redhorse (*Moxostoma duquesnei*), bluegill (*Lepomis macrochirus*), and redear sunfish (*Lepomis microlophus*) were significantly larger in open reaches, potentially reflecting differences in

Fig. 5. Frequency histogram of abundances of *Campostoma oligolepis* in open (open bars) and forested (solid bars) reaches grouped by standard length of individual fish (combined for all five sites).



food resources. Conversely, speckled madtom (*Noturus leptacanthus*) and *P. nigrofasciata* were significantly larger in forested reaches. *Fundulus stellifer*, Coosa banded sculpin (*Cottus carolinae zopherus*), and *L. auritus* were larger in forested reaches when YOY were included, and *H. etowanum* and speckled darter (*Etheostoma stigmaeum*) were larger in open reaches when YOY were excluded, suggesting that there were more YOY in open reaches for all five species (Appendix A). Size differences in *C. oligolepis* suggest that there were more, smaller individuals in open reaches, and for the first apparent age class, individuals were larger in open reaches than in forested reaches (Fig. 5).

Based on gut content analysis, we found no significant differences in the diversity of prey items in the guts of fish

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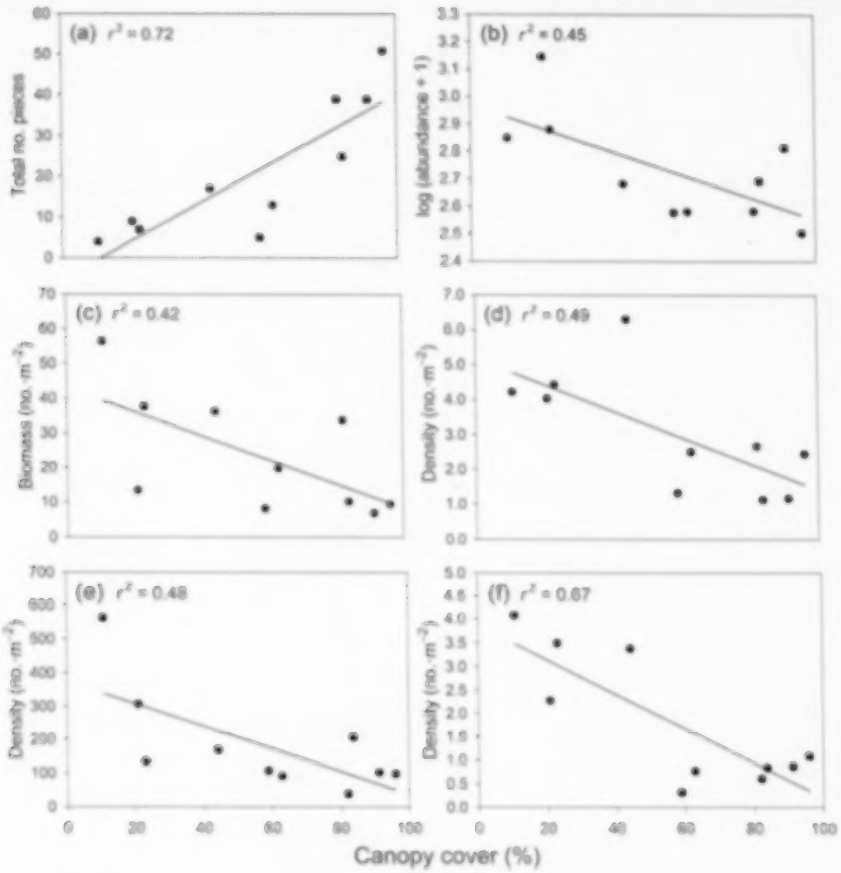
Fig. 6. Linear regressions (r^2) for selected environmental variables and percent canopy cover in 10 stream reaches: (*a*) large wood; (*b*) total fish species; (*c*) chlorophyll a; (*d*) habitat generalist fish species; (*e*) Ephemeroptera, Plecoptera, and Trichoptera invertebrate taxa; (*f*) herbivore fish species.



invertivores (*P. nigrofasciata* and *C. callistia*) or the trophic generalists (*L. auritus* and *H. etowanum*) (Appendix A). *Lepomis auritus* had higher proportional densities of dipteran pupae in open reaches (3.5% versus 0.5%, paired *t* test, $p = 0.015$, $n = 5$), higher arachnids in forested reaches (5.1% versus 3.4%, $p = 0.03$), and a trend toward higher densities of terrestrial prey in forested reaches (54% versus 44%, $p = 0.15$). Water column cyprinid fishes (*C. callistia*, two sites, and *N. xaenocephalus*, one site) contained over twice the proportional density of terrestrial prey in forested reaches (47% compared with 22% in open reaches, $p = 0.106$). Although we hypothesized that fishes would consume a higher proportion of scraping insects in open reaches, differences of aquatic mayflies (Ephemeroptera, primarily scrapers) in gut contents between open and forested reaches were inconsistent across the fish species examined and not statistically significant (all $p > 0.15$).

Relationships based on percent canopy cover

Although our site selection criteria resulted in clear differences between open and forested reaches within sites (smallest difference between canopy cover in open and forested reaches was 33.3% in Lawrence Creek) (Table 1), there was a wide range in canopy cover in open (10.6%–62.8%) and forested reaches (58.9%–96.1%) across sites. Thus, we examined relationships between percent canopy cover and environmental and biotic variables to see if differences were better explained by the gradient of forest canopy cover. For the variables with data from all 10 sites, six were significantly explained by percent canopy cover ($r^2 > 0.42$, $p < 0.05$) (Fig. 6). Examination of scatterplots suggests that the amount of large wood is directly related to canopy cover within the reach ($r^2 = 0.72$). However, percent canopy cover explains less than half of the variance (42%–48%) in chlorophyll *a*, Ephemeroptera, Plecoptera, and Trichoptera invertebrate density, and fish abundance, and observation of scatterplots verifies that canopy cover is not strongly driving these variables. The significant relationships between percent canopy cover and habitat generalist and herbivore fish species seem to be attributed to two separate groups with open (<50% cover) and forest canopies (>50% cover) (Fig. 6). In other words, except for Lawrence Creek where both reaches have relatively high forest cover, the difference in open and forest canopies in adjacent reaches within a

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stream better explains differences in environmental and biotic variables.

Discussion

Changes in stream quality and integrity

Habitat diversity, a measure commonly used to evaluate stream quality, was remarkably unaffected by reach-scale riparian deforestation in our study, suggesting that habitat quality may be controlled by catchment-scale factors (*e.g.*, land cover and hydrology) more than by reach-scale forest cover in these urbanizing streams. In fact, of 27 morphometric, bed texture, and habitat diversity (depth, velocity, and particle size) variables measured (Roy 2004), the only difference between reaches was in wetted width. Narrower channels in open (pasture) versus forested reaches have been found in several studies (Davies-Colley 1997; Scarsbrook and Halliday 1999; Sweeney et al. 2004); this is likely due to higher floodplain deposition rates relative to cutbank erosion in streams with grassy bank vegetation (Allmendinger et al. 2005). Because mean depth and baseflow discharge were not different between reaches, mean velocities were likely lower in forested reaches. Large wood is also important in structuring stream habitat, which was strongly explained by percent canopy cover, indicating that downstream transport of wood from forested to open reaches was not obscuring differences between reaches. Although local riparian deforestation may not eliminate large wood from stream systems, long-term reductions in supply might have an important influence on large wood volume and habitat complexity in urban streams.

Water quality was not expected to differ between open and forested reaches, since chemistry was assumed to be driven by catchment-scale factors such as urban and agriculture land cover and hydrologic alteration. Although most of the water quality variables did not differ between reaches, we measured higher amounts of daytime dissolved oxygen in open reaches. This difference is likely due to increased photosynthesis in open reaches that have higher amounts of primary producers. We also found higher concentrations of NO_2/NO_3-N in forested reaches. Although riparian zones are typically sinks for nitrate, Groffman et al. (2003) found that nitrification is increased in urban systems because of lowered water tables. Uptake of nitrogen by algae in open reaches may also be important in driving lower ambient NO_2/NO_3-N concentrations in open reaches. Local riparian deforestation increased summer minimum water temperatures by about 1 °C; however, we did not observe significant annual responses of temperature and temperature fluctuations to riparian deforestation. Longer openings in the riparian forest canopy are likely to alter stream temperatures more significantly, which may substantially impact fishes that cannot tolerate warmer temperatures (Scott and Helfman 2001).

Reach-scale biotic integrity was largely unaffected by local canopy cover. These streams had lower macroinvertebrate and Ephemeroptera, Plecoptera, and Trichoptera richness relative to other streams in the area; a random sample of 30 small (8–121 km^2) Etowah streams sampled in 1999 averaged 15% urban land cover (compared with 24.7% in this study) and over nine Ephemeroptera, Plecoptera, and Trichoptera species (compared with six in this study; Roy et al. 2003*b*). However, contrary to the expected reach-scale benefits of riparian forests to biotic integrity, we found no differences in macroinvertebrate assemblage integrity between open and forested reaches. This suggests that macroinvertebrate assemblages are likely driven more by catchment-scale factors (e.g., land cover) or reach-scale habitat quality than reach-scale forest cover. Salamander catches also did not differ based on forest cover, supporting the results of Hawkins et al. (1983) and Wilson and Dorcas (2003). Again, the reduced richness in these urban streams (only one species observed) may suggest that salamanders are primarily impacted by factors other than reach-scale riparian disturbance.

Total and endemic fish species richness did not indicate differences in fish assemblage integrity between reaches. Although fish assemblages had higher densities of tolerant and habitat generalist species in open reaches, these were driven by the abundance of two species (*C. oligolepis* and *F. stellifer*) and do not reflect an overall community shift in biotic integrity between reaches. The lack of response in biotic assemblages may be a consequence of minimal differences in habitat quality with reach-scale riparian deforestation. It is also possible that urban streams are already so depauperate of fishes that further changes in land cover would not affect the assemblage. However, our study streams supported 10–18 fish species, similar to fish richness (10–23) observed by Walters et al. (2003) in their 10 smallest Etowah streams. It is more likely that the high mobility of fishes and the presence of sufficient habitat and (or) thermal refugia in the sampled reach or adjacent reaches prevented severe local depletion of fish species. Given sufficient fish mobility, the open reaches could also be areas of "sink" fish populations supported by upstream or downstream forested areas (Gotelli and Taylor 1999; Jones et al. 1999).

Changes in the trophic basis of production

Reach deforestation resulted in reduced organic inputs into the stream. On average, the open reaches had one fifth the amount of trees compared with forested reaches, which is expected to translate into a significant reduction in leaf inputs. For example, in a study looking at the effects of forest clearcutting on a headwater stream in North Carolina, tree basal area was reduced from 23.7 to 7.9 $m^2 \cdot ha^{-1}$ (Elliot et al. 1997), and there was a concomitant reduction in litterfall (259.2–4.2 $g \cdot m^{-2}$) and blow-in (174.8–38.6 $g \cdot m^{-1}$) inputs to the stream (Webster and Waide 1982). We also found a direct relationship between canopy cover and large wood, resulting in a limited amount of allochthonous material and stable surface area for autochthonous production in open reaches.

As expected, we found a higher amount of chlorophyll *a* in open versus forested reaches, suggesting that light is limiting primary production in the forested reaches. We only found differences in biofilm biomass on rock substrates, emphasizing the importance of stable substrate for biofilm production in these streams. Differences in chlorophyll *a* and biofilm biomass potentially created an important difference in the trophic basis of production between reaches, which

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we predicted to transfer to higher trophic levels. There were trends toward higher overall densities of benthic invertebrates in open reaches; however, benthic invertebrate densities also were more variable in open reaches. Contrary to our hypotheses, we did not observe any differences in the densities of scraping or shredding invertebrates between reaches. Because we only sampled invertebrates in one season (spring), we were unable to determine whether differences existed at other times of the year when food resources may differ more between open and forested reaches (e.g., during autumn leaf-fall for shredders). The lack of differences in functional feeding groups may also be due to invertebrate consumption by higher trophic levels. However, there were no consistent differences in the proportion of scraping insects (Ephemeroptera) in the guts of insectivorous fishes or salamanders. The only evident response of consumers to the shift in basal resources was the significantly higher densities of herbivorous fishes (*C. oligolepis*) in open reaches, presumably driven by higher amounts of algae.

Terrestrial invertebrates are also important food resources for some insectivorous fishes, and inputs were predicted to differ between paired reaches. Contradicting our expectations, we found higher densities of terrestrial invertebrate inputs in open reaches; however, similar results were also seen by Romaniszyn (2000) in Appalachian streams with cleared riparian zones. Higher densities in our study were primarily attributed to increased inputs of small-bodied Diptera and Collembola in open reaches. Correspondingly, there were trends toward larger animals and higher terrestrial invertebrate biomass in forested reaches. Because drift-eating fish are visual predators and tend to select larger invertebrates for consumption (Zaret 1980), these differences in terrestrial inputs were expected to translate into differences in fish diets. Water column fishes, especially cyprinids, showed trends of higher percent terrestrial prey in forested reaches, but our small sample sizes did not result in statistical significance. While some studies have found reduced dependence on terrestrial organic matter associated with riparian deforestation (Hicks 1997; Hession et al. 2002; England and Rosemond 2004), other results suggest that annual prey consumption by fishes can be similar even with drastically different inputs of terrestrial invertebrates (Kawaguchi and Nakano 2001). This may result from high fish mobility or invertebrate drift from upstream patches. Because stomach contents do not account for variable digestion of prey or the relative incorporation of terrestrial- versus aquatic-derived carbon in the fish tissue, we could not make any definitive conclusions about the relative contribution of food items to fish biomass.

Comparisons of fish size revealed inconsistent effects of riparian cover on population structure or sizes of individuals. For eight species, there were higher abundances of YOY individuals in the open reaches, possibly reflecting availability of shallow habitat in open reaches or behavioral avoidance of larger individuals in forested reaches. Two benthic invertivores (*N. leptacanthus* and *P. nigrofasciata*) were larger in forested reaches, suggesting possible differences in biomass of benthic invertebrates between reaches. Conversely, an invertivore, two generalist predators, and the herbivore *C. oligolepis* were larger in open reaches, potentially reflecting higher nutritional quality and assimilation efficiency of algal-derived resources (Cummins 1974; Rosenfeld and Roff 1992). Food, habitat, and biotic interactions may all be important in explaining the distribution of adult and juvenile fishes.

Comparison with similar research on urban riparian reforestation

This study used an exploratory approach comparing numerous variables between open and forested reaches to identify characteristics of stream reaches that may differ based on riparian canopy cover in urbanizing streams. Because we performed numerous paired *t* tests and regressions, there is a risk of type I error associated with a cutoff p value of 0.05 (Zar 1974). However, owing to the small sample size and powerful parametric statistical analyses performed, there is also a risk of type II error, such that there may be differences that are not detected at a significance level of $p < 0.05$. Thus, these results must be taken strictly as evidence of possible differences (or lack thereof) that provide predictions for further testing in urban streams.

Recent research by Hession et al. (2003) in the Piedmont of southeast Pennsylvania and northern Delaware provides an opportunity to evaluate the generality and significance of our results (see also Sweeney et al. 2004). The Hession et al. (2003) study included 12 pairs of sites with forested and nonforested (open) canopies that ranged in land cover from 1% to 91% urban (mean = 56.3% urban) and had similar criteria for site selection as our study. Where data were comparable, there were no conflicting results between the two studies (Table 5). Both indicated that open reaches had lower tree density, but trees in open reaches were larger in diameter. In addition, both studies showed that streams were narrower in open reaches, and there were no differences in depth between reaches. Lower amounts of large wood were found in open reaches in both studies, and Hession et al. (2003) demonstrated that bank stability was reduced in open reaches relative to forested reaches (not measured in this study). Other measures of physical habitat, including variability in velocity, depth, and particle size, were not different between reaches in either study, suggesting that reach-scale canopies do not control local habitat diversity. Both studies found higher chlorophyll *a* concentrations in open reaches; however, there were no clear differences in benthic invertebrates between reaches. In terms of fishes, both studies found significantly higher densities in open reaches, including higher densities of tolerant fish species. Although the studies measured trophic composition in different ways, both demonstrated a trophic response to increased algal resources in open reaches, as indicated by increased herbivore densities in this study and increased $\delta^{13}C_{periphyton}$ in consumers in the Hession et al. (2003) study (Table 5). The consistent results in these two studies strengthen the conclusion that reach-scale riparian deforestation may result in trophic responses but has minimal effects on habitat quality and biotic integrity.

In this study, we primarily analyzed all biotic data in terms of relative abundances (unitless) and densities (i.e., per square metre of area) rather than per stream length because we felt that these were the most appropriate units for com-

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Table 5. Responses to reach-scale riparian deforestation in Piedmont streams of southeastern Pennsylvania and northern Delaware (n = 12 pairs) (Hession et al. 2003) and north-central Georgia (n = 5 pairs) (this study).

Response variable	Hession et al.	This study	Response variable	Hession et al.	This study
Riparian vegetation			Organic matter		
Tree density	↓	↓	Leaf inputs	↓	nd
Tree size	↑	Trend ↑	Large wood	↓	↓
Litter quality	—	nd	Algae		
Channel morphology			Biofilm biomass	↑	Trend ↑
Width	↓	Trend ↓	Chlorophyll *a*	↑	↑
Depth	—	—	Diatom composition	—	nd
Bed slope	—	—	Benthic intvertebrates[a]		
Physical habitat			Richness	nd	—
Rootwads	↓	nd	Density	nd	Trend ↑
Bank erosion rate	↑	nd	Terrestrial invertebrates		
Temperature	nd	Trend ↑	Density	nd	↑
Velocity variability	—	—	Biomass	nd	Trend ↓
Depth variability	—	—	Diversity	nd	↓
Particle size	—	—	Fishes		
Embeddedness	—	—	Density[b]	↑	↑
Nutrient concentrations			Biomass	↑	nd
SRP	↓	—	Tolerant	↑	↑
NH_4-N	Trend ↓	—	Herbivores	nd	↑
NO_2/NO_3-N	—	↓	Trophic composition		
DIN:SRP	↑	—	$\delta^{13}C_{periphyton}$	↑	nd
			% terrestrial in guts[c]	nd	Trend ↓

Note: An up arrow indicates significantly higher in open reaches ($p < 0.05$), a down arrow indicates significantly lower in open reaches ($p < 0.05$), trend indicates $0.05 < p < 0.20$, a dash indicates no difference ($p > 0.20$), and nd indicates no data. SRP, soluble reactive phosphorus; DIN, dissolved inorganic nitrogen.

[a]Hession et al. (2003) used a dissimilarity index to compare benthic invertebrate composition between forested and nonforested reaches and found no difference.

[b]Sweeney et al. (2004) reported significantly higher densities of benthic invertebrates in forested reaches and no differences in fish densities between reaches when calculated per metre of stream length (versus per m^2 surface area).

[c]*Lepomis auritus* and *Cyprinella callistia*.

paring adjacent stream reaches. Given the reach conditions, we found higher densities of fishes, terrestrial invertebrate inputs, and algal chlorophyll *a* in open reaches, suggesting that this reach could support more primary producers and consumers on per square metre basis. However, because streams were ~20% wider in forested reaches compared with open reaches, there may be differences in overall production and nutrient uptake potential per metre of stream length (Sweeney et al. 2004).

Implications for urban riparian reforestation

Although riparian buffers imply a goal of protecting stream ecosystems, local forested riparian areas are limited by their ability to mitigate upstream and downstream disturbances. Many studies have demonstrated the importance of catchment-scale land cover relative to local-scale land cover in driving habitat quality and biotic integrity (Richards et al. 1996; Roth et al. 1996; Allan et al. 1997). Although Roth et al. (1996) found that land cover within riparian areas was correlated with fish and habitat, these relationships only existed for riparian areas for the entire upstream network, not local riparian cover. This study directly tested changes associated with local riparian cover and also found that habitat quality and biotic integrity were not affected by local riparian deforestation. Although we were unable to test the relative importance of catchment-scale land cover (because of the small sample size of nested watersheds, n = 5), these results suggest that preservation of forested riparian fragments is not adequate to protect stream ecosystems.

Our results demonstrate that reach-scale openings in riparian canopies can create local patches of increased food resources. Algal resources may be important for supporting food webs in urban systems where terrestrial organic inputs are minimized and altered storm hydrology affects organic matter retention. Although we observed trends of higher benthic invertebrate densities and significantly higher fish densities in open reaches, biomass and productivity may be similar between reaches, especially when calculated per linear length of stream reach (i.e., accounting for wider forested reaches). Further, the switch from allochthonous- to autochthonous-supported food webs at the reach scale, particularly in smaller headwater streams, may have important implications in terms of altered ecosystem function in these naturally forested landscapes (Cummins 1974).

We caution against concluding that forested canopies are not important, since the role of catchment land cover and extent and continuity of riparian forests were not tested in this study. These sites had >50% forest in the 30-m riparian zone in the upstream drainage network, so we do not know how these urbanizing streams would respond to a greater or more

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complete loss of riparian cover and associated organic matter inputs. Other research shows that riparian cover in the upstream network is important for maintaining sensitive fishes, while local reductions in riparian forest cover increase species richness and abundance of tolerant fish (Roy 2004). Further studies addressing effects of local riparian forest cover with a range of network deforestation would be useful to understand the relative importance of upstream land cover in reach-scale stream conditions.

Previous studies have shown that over the long term, low levels of urbanization can negatively impact fish assemblages, structuring them toward more tolerant species that are habitat and trophic generalists (Weaver and Garman 1994; Wang et al. 2000; Walters et al. 2003). The maintenance of riparian forests has been successfully used to mediate the negative effects of agricultural and forestry practices on aquatic ecosystems; however, the relative benefits of riparian reforestation may be reduced in watersheds with increasing urbanization. In urban streams, alteration of stormwater hydrology (because of increases in impervious surfaces and number of stormwater pipes connected to streams) is thought to have an overarching influence on stream ecosystem integrity (Paul and Meyer 2001; Walsh 2004). Further, many urban streams are channelized and extensively piped (although not the case in these study streams), thus drastically altering stream habitat condition (Paul and Meyer 2001). Although we did not test the function of reach-scale riparian forests along a gradient of urbanization, our study suggests that at low levels of urbanization, reach-scale riparian forests provided minimal benefits to reach-scale stream condition. The combined results of this study and the Hession et al. (2003) study provide strong evidence that although riparian forest cover may be an important regulator of stream width, temperature, and the food/energy base at the reach scale, it does not dictate local stream habitat quality or biotic integrity. Thus, reach-scale riparian reforestation is necessary but not sufficient to protect aquatic resources in urbanizing landscapes. Watershed management, including stormwater management, is also needed to mitigate the impacts of urbanization.

Acknowledgements

The authors thank the numerous technicians and volunteers for their assistance in field work throughout this project. Fishing field assistants included J. Diez, L. England, C. Gibson, I. Giladi, E. Green, M. Hagler, S. Herbert, K. Jones, M. Laurita, S. Madson, J. Shields, L. Skelton, L. Stephens, C. Storey, E. Sutherland, and R. Weyers. Salamanders were collected and analyzed for gut content analysis with the help of J. Williams. J. Boring, W. Clark, E. Curry, K. Jones, K. Loggins, and J. Shields assisted with other portions of the field work. K. Loggins sorted aquatic macroinvertebrate samples, and L. Faust assisted in sorting and identifying terrestrial invertebrates. Comments from L. England, L. Fowler, D. Leigh, J.B. Wallace, L. Johnson, and an anonymous reviewer greatly improved this manuscript. This project was primarily funded by a grant to A.H.R. from the Procter & Gamble Company through the North American Benthological Society. The University of Georgia Research and Engineering Apprenticeship Program awarded to C.L.F. provided funding for supplies for the terrestrial invertebrate portion. Additional support was provided to A.H.R. by the University of Georgia Research Foundation.

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Appendix A

Table A1 follows (pp. 2328–2329).

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Table A1. List of all fish species collected in the study, their feeding guild, and their sensitivity categories.

				All individuals					
				Open		Forest			
Family and scientific name	Common name	Feeding guild	Sensitivity	*n*	Length (mm)	*n*	Length (mm)	*t*	*p*
Petromyzontidae									
Ichthyomyzon gagei	Southern brook lamprey	H		2	115	9	141	—	—
Cyprinidae									
Campostoma oligolepis	Largescale stoneroller	H	C, T, G	1060	**59**	440	53	7.35	<0.001
Cyprinella callistia[a]	Alabama shiner	I	T, G	166	34	59	**55**	7.36	<0.001
Cyprinella venusta[a]	Blacktail shiner	I		47	24	22	**76**	14.04	<0.001
Hybopsis sp. cf. *winchelli*	Clear chub	BI		5	56	4	56	0.54	0.310
Notropis longirostris	Longnose shiner	I		107	26	8	33	1.69	0.065
Notropis stilbius[a]	Silverstripe shiner	I		107	28	17	**47**	5.24	<0.001
Notropis xaenocephalus[a]	Coosa shiner	I	E	257	42	230	**47**	4.86	<0.001
Phenacobius catostomus	Riffle minnow	BI	E	1	80	0	—	—	—
Semotilus atromaculatus	Creek chub	TG	C, T	43	87	54	86	0.11	0.458
Catostomidae									
Hypentelium etowanum	Alabama hog sucker	TG	T, G	210	83	194	81	0.72	0.236
Moxostoma duquesnei	Black redhorse	TG	C	52	**149**	18	113	3.00	0.002
Moxostoma poecilurum	Blacktail redhorse	TG	C	4	166	0	—	—	—
Ictaluridae									
Ameiurus brunneus	Snail bullhead	TG	C, G	1	145	1	56	—	—
Ameiurus natalis	Yellow bullhead	TG	C	1	127	0	—	—	—
Noturus leptacanthus	Speckled madtom	BI	C	69	42	17	**47**	1.87	0.036
Fundulidae									
Fundulus stellifer[b]	Southern studfish	TG		768	36	201	**54**	12.29	<0.001
Poeciliidae									
Gambusia affinis	Western mosquitofish	I	C, T	0	—	1	27	—	—
Cottidae									
Cottus carolinae zopherus	Coosa banded sculpin	BI	E	13	47	25	**52**	1.74	0.048
Centrarchidae									
Lepomis auritus	Redbreast sunfish	TG	C, T	169	62	168	**70**	2.93	0.002
Lepomis cyanellus	Green sunfish	TG	C, T	41	62	108	67	1.30	0.099
Lepomis gulosus	Warmouth	TG	C	0	—	1	80	—	—
Lepomis macrochirus	Bluegill	TG	C, T	251	**63**	365	58	4.04	<0.001
Lepomis microlophus	Redear sunfish	I	C	10	**68**	6	49	2.01	0.036
Micropterus coosae	Redeye bass	GC		10	78	14	96	1.27	0.108
Micropterus punctulatus	Spotted bass	GC	C	15	60	29	57	0.75	0.229
Micropterus salmoides	Largemouth bass	GC	C	19	96	15	69	1.49	0.073
Pomoxis nigromaculatus	Black crappie	TG	C	0	—	1	110	—	—
Percidae									
Etheostoma scotti	Cherokee darter	BI	E	157	42	128	41	0.49	0.313
Etheostoma stigmaeum	Speckled darter	BI	C	20	42	22	40	1.33	0.096
Perca flavescens	Yellow perch	TG	C, G	1	166	0	—	—	—
Perca kathae	Mobile logperch	BI		11	85	2	80	—	—
Perca nigrofasciata	Blackbanded darter	BI	C, T	99	48	63	**59**	4.36	<0.001

Note: Comparison of mean fish standard lengths between open and forested reaches (two-sample *t* test assuming unequal variances) for all individuals. I, invertivore; BI benthic invertivore; TG, trophic generalist; GC, generalized carnivore. Sensitivity categories: E, endemic; C, cosmopolitan; T, tolerant; ($p < 0.05$). Species with less than four individuals in one or both reaches were not analyzed (indicated by a dash). *n* is the number of individuals.

[a]Difference in length because of YOY fishes in the open reach of Weatbrook Creek.

[b]Difference in length because of YOY fishes in the open reaches of multiple streams.

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YOY excluded					
Open		Forest			
n	Length (mm)	*n*	Length (mm)	*t*	*p*
2	115	9	141	—	—
755	65	147	**71**	4.46	<0.001
34	61	40	**66**	1.98	0.026
3	66	21	78	—	—
5	56	4	56	0.535	0.310
11	51	3	46	—	—
9	61	13	54	1.44	0.090
109	52	173	52	0.42	0.338
1	80	0	—	—	—
30	104	41	99	0.9	0.187
88	**120**	97	109	3.33	<0.001
39	**175**	11	140	4.45	<0.001
4	166	0	—	—	—
1	145	1	56	—	—
1	127	0	—	—	—
62	44	15	**49**	2.59	0.007
138	69	120	68	1.07	0.142
0	—	1	27	—	—
5	55	19	55	0.04	0.484
112	75	144	75	0.18	0.429
35	65	97	69	1.09	0.141
0	—	1	80	—	—
221	**67**	285	63	3.23	<0.001
9	**71**	5	50	2.01	0.040
6	99	10	115	1.41	0.093
0	—	1	126	0.749	0.229
7	164	4	118	1.37	0.102
0	—	1	110	—	—
157	42	125	42	0.125	0.450
17	**45**	21	40	3.22	0.001
1	166	0	—	—	—
11	85	2	80	—	—
72	55	56	**62**	2.98	0.002

and with YOY individuals excluded. Feeding guilds: H, herbivore; G, habitat generalist. Bold numbers indicate significantly longer lengths.

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Foraging by a young-of-the-year piscivore: the role of predator size, prey type, and density

Tracy L. Galarowicz and David H. Wahl

Abstract: Young-of-the-year piscivores undergo ontogenetic diet shifts, but mechanisms influencing prey selection and implications for growth are unclear. We examined foraging and growth of 20- to 150-mm walleye (*Sander vitreus*) fed either zooplankton, benthic invertebrates, or fish over a range of prey densities in the laboratory. The number of each prey type consumed was influenced by walleye size and prey density. Walleye exhibited type II functional responses on each prey type; attack coefficients were constant across zooplankton and fish densities but decreased with benthic invertebrate densities. Handling time estimates were greater for fish than for other prey types but similar for zooplankton and benthos. Foraging efficiencies on zooplankton and benthic invertebrates increased with walleye size but were variable for fish prey. The smallest walleye size class (20 mm) had similar energy return ($J \cdot min^{-1}$) and growth ($g \cdot day^{-1}$) on zooplankton, benthic invertebrates, and fish. For larger walleye, both energy return and growth were highest on fish, intermediate on benthic invertebrates, and lowest on zooplankton. Diet shifts of juvenile piscivores and, consequently, growth can be explained by ontogenetic changes in foraging abilities and prey densities.

Résumé : Les jeunes piscivores de l'année subissent un changement ontogénique de régime alimentaire; cependant, les mécanismes qui influencent le choix des proies et les conséquences du changement sur la croissance restent mal connus. Nous avons étudié en laboratoire l'alimentation et la croissance de dorés (*Sander vitreus*) de 20–150 mm de longueur nourris de zooplancton, d'invertébrés benthiques ou de poissons à diverses densités de proies. Le nombre de proies consommées de chaque type dépend de la taille des dorés et de la densité des proies. Les dorés ont une réponse fonctionnelle de type II en présence de toutes les proies; les coefficients d'attaque restent constants à toutes les densités de zooplancton et de poissons, mais ils décroissent en fonction des densités d'invertébrés benthiques. Le temps estimé requis pour la manipulation est plus grand pour les poissons que pour les autres types de proies, mais il est le même pour le zooplancton et le benthos. L'efficacité de l'alimentation augmente en fonction de la taille des dorés dans le cas du zooplancton et des invertébrés benthiques, mais elle est variable en ce qui a trait au poissons. Les dorés de la plus petite classe de taille (20 mm) ont des bénéfices énergétiques ($J \cdot min^{-1}$) et des croissances ($g \cdot jour^{-1}$) semblables, qu'ils se nourrissent de zooplancton, d'invertébrés benthiques ou de poissons. Chez les dorés plus grands, le bénéfice énergique et la croissance sont tous deux plus élevés avec un régime de poissons; ils sont moyens avec un régime d'invertébrés benthiques et plus bas avec un régime de zooplancton. Les changements de régime alimentaire chez les jeunes piscivores et, par conséquent leur croissance, peuvent s'expliquer par des changements ontologiques dans leur capacité à s'alimenter et par les densités de proies.

[Traduit par la Rédaction]

Introduction

Ontogenetic niche shifts are defined as a change in habitat or resource use as an organism increases in size (Werner and Gilliam 1984). Size-related diet shifts may be the result of differences in susceptibility to predators (Werner et al. 1983; Werner and Hall 1988; Dahlgren and Eggleston 2000). Alternatively, size-specific changes in foraging ability allow individuals to successfully capture more energetically beneficial prey types and are often correlated with discrete periods of growth that may occur at critical periods in the life history of fish (Hokanson and Lien 1986; Wicker and Johnson 1987; Buijse and Houthuijzen 1992). Predicting ontogenetic diet shifts requires information on costs and benefits associated with each prey type across a range of predator sizes. Although few studies directly measure the growth consequences of diet selection (Persson 1990), prey profitability should be positively related to growth for different prey types.

Optimal foraging models are based on the assumption that animals maximize energy intake while minimizing costs associated with searching and handling prey (Stephens and

Received 19 June 2004. Accepted 24 April 2005. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on 23 September 2005.
J18177

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 doi: 10.1139/F05-148 © 2005 NRC Canada

Krebs 1986). These models are often employed to predict diet selection given characteristics of the predator and prey and can successfully predict resource and habitat use (Mittelbach 1981; Werner et al. 1983). However, these models tend not to describe the mechanisms involved in acquiring the prey (Stephens and Krebs 1986). Because these models assume that the forager has knowledge of available prey profitabilities, discrepancies between actual prey selection and model predictions can occur. In addition, when predators include a variety of prey types in their diet that vary greatly in habitat and behavior, larger discrepancies may occur.

Size-specific attributes of predators, characteristics of prey, and prey densities can influence diet shifts. Prey selection can be constrained by mouth gape for a variety of prey types including zooplankton (Bremigan and Stein 1994; Mayer and Wahl 1997) and fish (Johnson 1969; Hambright 1991; Reimchen 1991), and encounter rates with prey increase with size-related changes in swimming speeds and reactive distances (Gerritsen and Strickler 1977; Breck and Gitter 1983). Zooplankton, benthic invertebrates, and fish may all be susceptible to juvenile fish predators, but each of these general prey types varies in size, shape, behavior, and habitat. In addition, prey densities influence encounter rates and search times by predators (Mathias and Li 1982; Townsend and Winfield 1985; Donovan et al. 1997) and may vary dramatically over relatively short periods of time. For example, in midwestern lakes, zooplankton densities peak in late spring and early summer (Claramunt and Wahl 2000), whereas benthic invertebrate densities remain relatively constant (Kline 2000). Larval fish densities vary among species but generally peak midsummer (Claramunt and Wahl 2000). Complex interactions among prey types and densities likely influence timing of ontogenetic diet shifts.

Juvenile piscivores typically undergo diet shifts switching from invertebrates (zooplankton and macroinvertebrates) to fish; these shifts vary across systems depending on predator size and prey availability (Olson 1996; Robichaud-LeBlanc et al. 1997). Young-of-the-year walleye (*Sander vitreus*) are initially zooplanktivorous, switch to benthivory, and finally become piscivorous (Mathias and Li 1982; Fox 1989; Madon and Culver 1993). The onset of piscivory typically occurs for walleye between 50 and 80 mm (Smith and Moyle 1945; Smith and Pycha 1960; Walker and Applegate 1976) but has been observed in larval walleye (Li and Mathias 1982) and walleye as large as 100 mm (Li and Ayles 1981; Kolar et al. 2003). Factors responsible for this variability in walleye switches to piscivory are unknown. Although prey selection by larval walleye (Johnston and Mathias 1994; Mayer and Wahl 1997) and juvenile walleye (Mathias and Li 1982) on zooplankton has been examined extensively, foraging behaviors by young-of-the-year walleye on all three prey types at different prey densities have not been examined. Additionally, the effect of these diet shifts among prey types on growth of walleye is unknown but may be predictable using an optimal foraging framework.

In this study, we examine how young-of-the-year walleye size and prey densities influence consumption of three types of prey: zooplankton, benthic invertebrates, and fish. These three prey types represent the major items found in walleye diets throughout the year. We investigate how foraging behaviors differ with density and size with the goal of understanding the factors that drive consumption of each prey type. We examine the effect of prey density by determining functional response relationships for each prey type over a range of walleye sizes. With information gained from observing walleye foraging behavior, we predict energy return on each prey type and compare it with growth over a range of walleye sizes. By examining the consequences of foraging on each prey type, we can link ontogenetic foraging patterns to prey type, densities, profitability, and growth.

Methods

Two different experiments were conducted in the laboratory to examine ontogenetic changes in walleye foraging and growth on three different prey types (zooplankton, benthic invertebrates, and fish). Experimental walleye were fed ad libitum all three prey types daily at least 2 weeks before both experiments to ensure equal experience with each group. We observed walleye of all sizes consuming each of the prey types during the acclimation period. In the first set of experiments, walleye of 10 different size classes (20–150 mm) were allowed to forage on one of the prey types at different densities. Foraging behaviors were observed for 15 min, and the parameters of a Holling type II functional response model were estimated for each size class – prey type combination. In the second experiment, growth was measured for five different walleye size classes (20–100 mm) fed one of the three prey types ad libitum for 7 days. Details of each experiment are given below.

Foraging experiments

We examined the effect of different prey types and densities on consumption and foraging behaviors by 20, 30, 40, 50, 60, 70, 80, 90, 100, and 150 mm size classes of age-0 walleye. The walleye were obtained from the Jake Wolf Memorial Fish Hatchery, Mankito, Illinois, as either larvae (9 mm) or juveniles (35 mm). Walleye were then held in the laboratory at the Kaskaskia Biological Station, Illinois Natural History Survey, Sullivan, Illinois, and grown to appropriate sizes for the experiments. Water temperatures were maintained at 21 °C (±0.05 SE).

Walleye were offered either zooplankton (1–100·L^{-1}), benthic invertebrates (25–1000·m^{-2}), or fish (13–93·m^{-3}) at six different prey densities (see Fig. 1 for prey densities). Densities were selected to encompass the range found in natural systems for each prey type (Welker et al. 1994; Claramunt and Wahl 2000; Kline 2000). The prey used in all experiments were either cultured in the laboratory or collected from local ponds and reservoirs. Zooplankton (*Daphnia* spp.) was sieved (63-μm mesh) to isolate individuals of similar size. Subsamples were preserved in 95% ethanol and total length (mm) was measured in the laboratory using a dissecting microscope and digitizing pad (mean = 1.64 mm, range = 0.67–2.60 mm, SE = 0.02 mm). Zooplankton were hand-counted using a dissecting microscope, but because this was time intensive, volumetric subsamples were used for the two highest densities. As with the zooplankton, similar sizes of benthic invertebrates (chironomid larvae) were used, and subsamples were measured in the laboratory (mean = 11.35 mm, range = 7.07–16.31 mm, SE =

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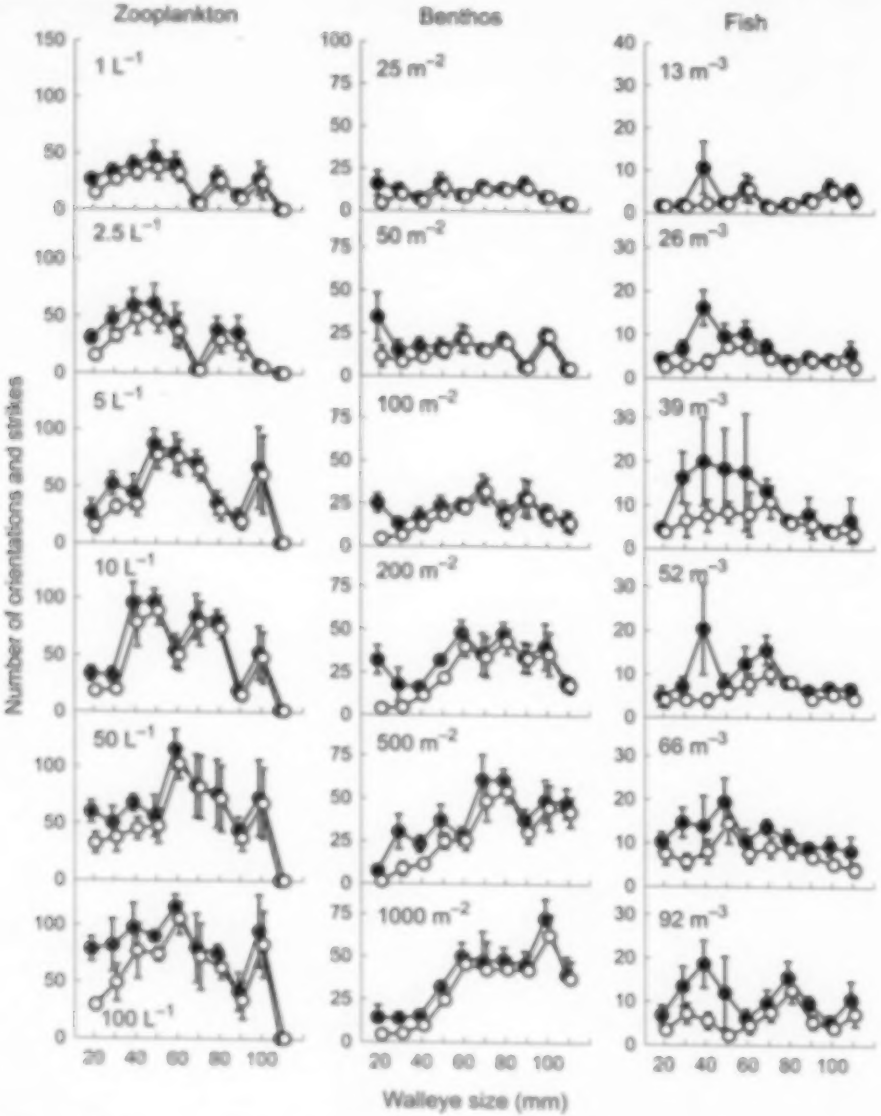
Fig. 1. Number of orientations (solid circles) and strikes (open circles) on zooplankton, benthic invertebrates, and fish for different densities of each prey type as a function of walleye (*Sander vitreus*) size (20–150 mm). Note that the *y* axis is on a different scale for each prey type. Values are means ± 1 SE.



0.33 mm). Small walleye (20–30 mm) were fed larval common carp (*Cyprinus carpio*), whereas larger walleye were fed bluegill (*Lepomis macrochirus*) owing to timing of availability. Common carp and bluegill are physically similar as larvae (Auer 1982) and have poorly developed antipredator behaviors at these sizes. Weights (g) and lengths (mm) of the prey fish were taken prior to each experimental trial. Fish length was maintained at 25%–30% of the walleye total length because fish prey size can affect consumption (Einfalt and Wahl 1997) and the lengths of available prey fish would increase throughout the season in natural systems.

Consumption and foraging behaviors of walleye for each treatment were observed in aquaria. Experimental tanks were 72-L aquaria with an opaque divider separating the tanks into a 21-L acclimation chamber and 51-L experimental chamber. Three sides of the tank were covered with black plastic to reduce disturbance to the walleye and prey, whereas one side was left uncovered for observations. The tanks were filled with filtered water and a fine sand substrate (1 cm in depth). Walleye were starved 24 h to ensure similar hunger levels and placed into the acclimation chamber for at least 1 h prior to the start of a trial. One of the prey types was then added at a specified density. Zooplankton were introduced into the experimental chamber 5 min before the trial to avoid unintentional movement of the zooplankton into the acclimation chamber, benthic invertebrates were added 1 h before the trial to allow the larvae to burrow and acclimate

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to the substrate, and prey fish were introduced 15 min before the start of the experiment. After acclimation, the divider was raised allowing the walleye access to the prey; initiation of the trial began with movement of the walleye into the experimental chamber. Foraging behaviors were observed for 15 min (or until consumption of the last prey item was complete) and recorded on audiotape for further analysis; at the end of the experiment, walleye were weighed (g) and measured (mm). For each prey type, at least three (range = 3–10, mean = 5.3, SE = 0.07) replicates were conducted for each walleye size at each prey density. A total of 226 walleye were used in the experiments. Walleye were randomly assigned to treatments, and while individual walleye may have been tested repeatedly, no walleye was used more than once per treatment.

Foraging behaviors were recorded as follows: search (actively swimming), orientation (orient body toward prey after encounter), pursuit, reject (neither pursue nor strike after initial orientation or pursuit), strike (attempt to capture prey), capture, ingest, and expulsion (O'Brien 1979). Consumption was defined as successful captures in which prey were not expelled. Overall effects of walleye length and prey density for each prey type on the total number of orientations, pursuits, strikes, and captures were examined using multivariate analysis of variance (MANOVA). When significant multivariate effects were found, we then examined the univariate responses for significant effects. Consumption (or ingestion) of each prey type was assessed by two-way analysis of variance (ANOVA) with walleye length and prey density as the main effects with a sequential Bonferroni adjustment (Rice 1989). We also calculated foraging efficiencies of the predation sequence: pursuits per orientation, strikes per pursuit, captures per strike, and ingestions per capture (see Holling 1966; Kolar and Wahl 1998). Behavioral probabilities were analyzed using MANOVA followed by individual univariate analyses with a Bonferroni sequential adjustment (Rice 1989). All analyses were completed using PROC GLM (α = 0.05) in SAS (SAS Institute Inc. 2001). If a significant interaction between walleye length and prey density was found, these relationships were investigated further. Data were log transformed to homogenize variances (Zar 1999), although untransformed means are presented graphically.

Functional responses

We first used logistic regression to assess the relationship between the proportion of prey consumed and the number of prey available. These analyses were used to determine the appropriate functional response model to represent our data (Trexler et al. 1988; Juliano 1993; Trexler and Travis 1993). For each walleye size – prey density combination, Holling's type II functional response best described the relationship between the number of each prey type eaten for different walleye sizes. Type II relationships were determined to be appropriate for our data after comparing type II and III curves (Trexler et al. 1988; Juliano 1993). Holling's type II functional response is defined as

$$(1) \qquad N_{\text{eaten}} = aT_t N/(1 + aT_h N)$$

where a is the attack coefficient (attacks per second), T_t is the total time available for foraging (seconds), T_h is the handling time per prey, and N is the prey density (Holling 1959). Parameter T_h includes all time spent pursuing, capturing, and consuming prey plus time preparing to search for the next prey item (Holling 1966). Parameter estimates of a and T_h (±1 SE) were obtained using Newton's method of nonlinear regression (PROC NLIN) (SAS Institute Inc. 2001), and R^2 values were computed (Zar 1999). Because prey availability decreased during the experiment, prey depletion was incorporated into the analysis (Juliano 1993). We used analysis of covariance to test differences in a and T_h between prey types using walleye length as a covariate (Zar 1999). Data were log transformed to homogenize variances (Zar 1999).

Energy return

Energy return (joules per minute) was calculated as

$$(2) \qquad \frac{En}{T} = \frac{\lambda_{ij} E_i P_{ij}}{1 + \lambda_{ij} T_{hij} P_{ij}}$$

for prey type i and walleye size j. Parameter λ_{ij} is the number of orientations towards prey i by walleye size j during the feeding trials (sensu Charnov et al. 1976) as determined in the feeding experiments. Parameter E_i is the expected energy gain (J) per individual prey item and was calculated by multiplying the biomass of an individual prey item (g) by the energy density (J·g^{-1}). Length–weight regressions were used to estimate biomass for zooplankton (daphnids; Culver et al. 1985; Dumont et al. 1975) and chironomids (Smock 1980); mean fish weights were determined directly (nearest 0.01 g) for each walleye size class. We used energy density estimates from Cummins and Wuycheck (1971): 1314 J·g wet weight^{-1} for zooplankton, 2746 J·g wet weight^{-1} for benthic invertebrates (dipteran larvae), and 6250 J·g wet weight^{-1} for fish. Parameter P_{ij} is the probability of an attack occurring after orientation and was determined by multiplying the probability of pursuit (pursuits per orientation) by the probability of striking (strikes per pursuit). Parameter T_{hij} is the handling time for each prey type i for walleye size j as determined by the functional response relationships (eq. 1). We assumed that energy is maximized when feeding reaches an asymptote, and as a result, we calculated energy return for only one density per prey type near where consumption stopped increasing: zooplankton at 50·L^{-1}, benthic invertebrates at 500·m^{-2}, and fish at 66·m^{-3}. The influence of walleye length and prey density on energy return was assessed using two-way ANOVA (α = 0.05) (PROC GLM) (SAS Institute Inc. 2001).

Walleye growth

We examined the effects of the different prey types on relative growth of 20-, 40-, 60-, 80-, and 100-mm size groups of walleye. Appropriate sizes of walleye were placed in separate aquaria (one walleye per tank) and fed zooplankton, benthic invertebrates, or fish ad libitum for 7 days. Growth (g·day^{-1}) was determined as the weight gained divided by the length of the experiment (7 days). At least four (range = 4–7, mean = 5.00, SE = 0.22) replicates were conducted for each size class and prey type. Overall effects of walleye length and prey type on relative growth were examined using two-way ANOVA, and differences within each size class were tested with ANOVA and Tukey's honestly significant difference (α = 0.05) (SAS Institute Inc. 2001). The relation-

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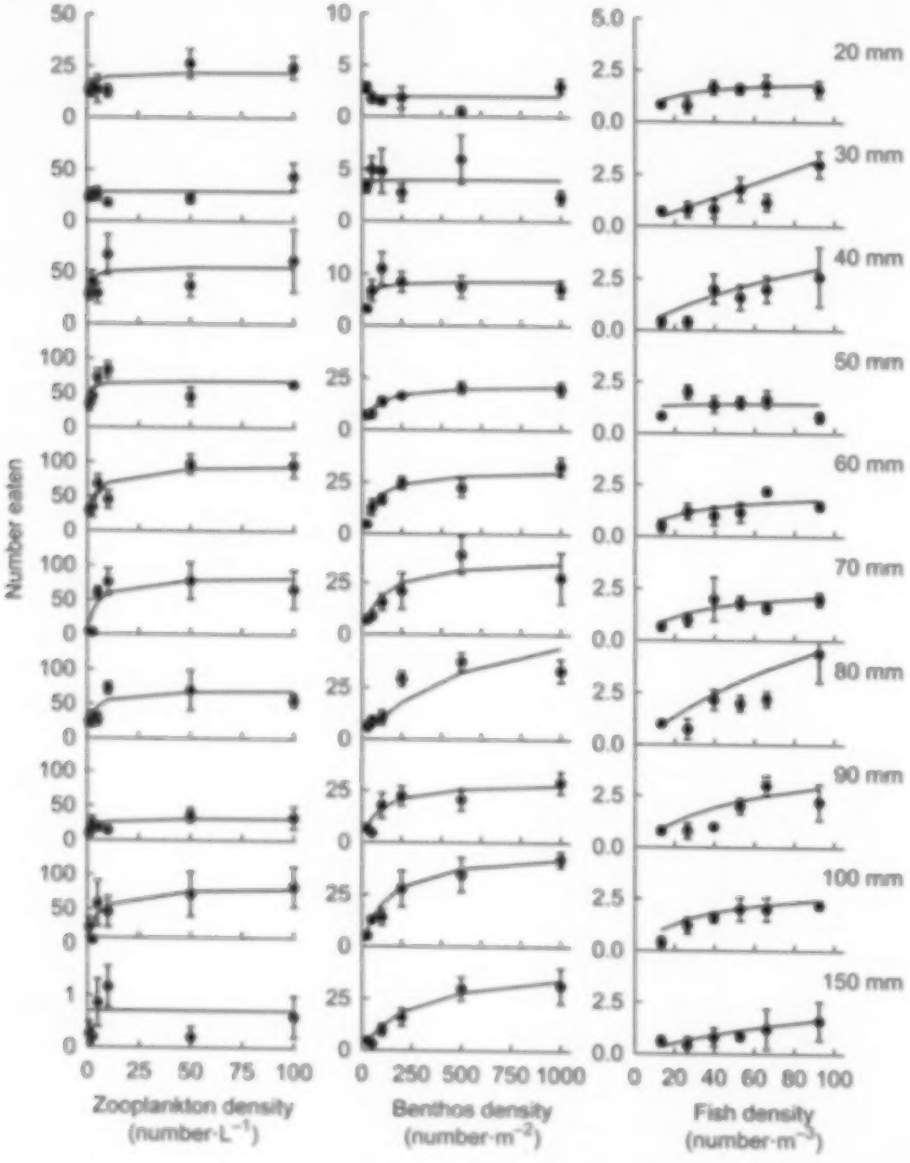
Fig. 2. Number of zooplankton, benthic invertebrates, and fish consumed by each walleye (*Sander vitreus*) size group as a function of prey density in feeding experiments. Mean number consumed is represented by circles, and the vertical bars represent ±1 SE. Fitted lines are type II functional response relationships. Attack coefficients, handling times, and R^2 values for the functional response curves are given in Table 3.



ship between growth and energy return was examined for each prey type by correlation (PROC CORR) (SAS Institute Inc. 2001).

Results

Foraging and behaviors

Prey density and walleye size influenced consumption (or successful captures) of each prey type (Fig. 2). The number of zooplankton consumed was significantly influenced by length ($F_{[9,255]}$ = 35.68, P = 0.0001), density ($F_{[5,255]}$ = 11.74, P = 0.0001), and their interaction ($F_{[45,255]}$ = 1.51, P = 0.03). The effect of zooplankton density was more pronounced for larger walleye with the exception of the 150-mm walleye, which consumed almost no zooplankton. As with zooplankton, benthic invertebrate consumption was significantly influenced by walleye size ($F_{[9,260]}$ = 32.34, P = 0.0001), prey density ($F_{[5,260]}$ = 29.59, P = 0.0001), and their interaction ($F_{[45,260]}$ = 2.52, P = 0.0001). As both length and density increased, the number of benthic invertebrates con-

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Table 1. Effect of walleye (*Sander vitreus*) length, prey densities, and their interaction on individual walleye feeding behaviors in aquaria experiments.

(*a*) MANOVA tests of effects of walleye length and prey density.

	Zooplankton			Benthic invertebrates			Fish		
Source	Pillai's trace	*F* (df)	*P*	Pillai's trace	*F* (df)	*P*	Pillai's trace	*F* (df)	*P*
Length	1.45	2.88 (45,1275)	0.0001	0.92	6.52 (45,1300)	0.0001	0.46	2.88 (45,1285)	0.0001
Density	0.33	4.07 (25,1275)	0.0001	0.62	7.30 (25,1300)	0.0001	0.37	4.07 (25,1285)	0.0001
Interaction	0.85	1.05 (225,1275)	0.31	1.01	1.46 (225,1300)	0.0001	0.78	0.78 (225,1285)	0.31

(*b*) ANOVA tests of the effects on individual walleye behaviors for significant terms in MANOVA.

	Model		Length		Density		Length × density	
Behavior	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Zooplankton								
Orientations	9.10	0.0001	43.02	0.0001	11.71	0.0001		
Pursuits			37.07	0.0001	10.71	0.0001		
Strikes			35.92	0.0001	11.38	0.0001		
Captures			35.31	0.0001	11.14	0.0001		
Benthic invertebrates								
Orientations	4.48	0.001	4.93	0.001	24.13	0.001	1.86	0.002
Pursuits			16.01	0.001	16.95	0.001	2.18	0.001
Strikes			21.79	0.001	16.60	0.001	2.26	0.001
Captures			26.28	0.001	20.72	0.001	2.20	0.001
Fish								
Orientations	2.71	0.01	3.14	0.01	15.47	0.0001		
Pursuits			2.72	0.01	14.10	0.0001		
Strikes			2.46	0.02	11.79	0.0001		
Captures	1.93	0.01	1.44	0.17	9.21	0.0001		

Note: Walleye (20–150 mm) were fed zooplankton, benthic invertebrates, or fish over a range of prey densities. Sums of squares are type III (SAS Institute Inc. 2001).

sumed increased, and effects of benthos density were most pronounced for larger sizes of walleye. In general, fewer benthic invertebrates were consumed compared with zooplankton. In contrast, the number of fish consumed was significantly influenced by both walleye size ($F_{[9,256]} = 2.47$, $P = 0.01$) and prey density ($F_{[5,256]} = 29.59$, $P = 0.0001$) but not by their interaction ($F_{[45,256]} = 0.99$, $P = 0.5$). Consumption increased with density, and there was a slight increase in the number consumed with length.

Foraging behaviors were also significantly influenced by walleye size and prey density (Table 1). Zooplankton density and size, but not their interaction, influenced individual foraging behaviors (Table 1). Orientations on zooplankton increased with length through 60 mm and then slowly declined, dropping sharply with the 150-mm walleye; orientations also increased with density (Fig. 1). Pursuits, strikes, and captures of zooplankton exhibited similar patterns; all of these behaviors increased with zooplankton density and peaked with walleye size between 40 and 60 mm (Fig. 1). In contrast, behaviors of walleye feeding on benthic invertebrates were influenced by size, density, and their interaction (Table 1). Orientations on chironomids increased with walleye size and prey density (Fig. 1); as density increased, the influence of size was more apparent. Pursuits, strikes, and captures of benthic prey followed similar patterns, increasing with prey density, walleye size, and their interaction (Fig. 1). Walleye length and prey density influenced most foraging behaviors with fish prey, but there was no interaction between these two factors (Table 1). Orientations increased with fish density and walleye size through 40 mm and then decreased gradually (Fig. 1). Pursuits and strikes increased slightly with density and peaked with size between 40 and 80 mm. However, captures increased with density but not with walleye size.

Foraging efficiencies varied with walleye length for all three prey types (Table 2). Probability of pursuit after orientation for zooplankton increased with size generally through 60–70 mm and then decreased for larger sizes but was influenced by neither zooplankton density nor an interaction between density and length (Fig. 3). Strike, capture, and ingestion efficiencies increased slightly with size and remained near 100% for large walleye but did not vary with density or an interaction (Figs. 3 and 4). Both walleye size and prey density, but not their interaction, influenced foraging efficiencies on benthic invertebrates (Table 2). For most behaviors, 20- and 30-mm walleye generally had lower efficiencies with benthic invertebrates than with zooplankton. Pursuit efficiency increased from about 43% for 20 mm to greater than 90% for walleye 60 mm and larger and then decreased slightly at higher densities (Fig. 3). Strike and capture efficiencies increased with walleye size and reached near 100% by 40 mm (Figs. 3 and 4); in fact, capture effi-

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Table 2. Effect of walleye (*Sander vitreus*) length, prey densities, and their interaction on individual walleye feeding behaviors.

(*a*) MANOVA tests of walleye length and prey density influence on walleye behavioral probabilities.

	Zooplankton			Benthic invertebrates			Fish		
Source	Pillai's trace	*F* (df)	*P*	Pillai's trace	*F* (df)	*P*	Pillai's trace	*F* (df)	*P*
Length	0.98	9.21 (36,1020)	0.0001	0.76	6.83 (36,1040)	0.0001	0.25	1.92 (36,1028)	0.0001
Density	0.11	1.50 (20,1020)	0.07	0.20	2.71 (20,1040)	0.0001	0.14	1.89 (20,1028)	0.01
Interactions	0.61	1.02 (180,1020)	0.40	0.66	1.14 (180,1040)	0.11	0.62	1.04 (180,1028)	0.36

(*b*) ANOVA tests of the effects of walleye length and prey density on walleye behavioral probabilities.

	Pursuits/orientation		Strikes/pursuit		Captures/strike		Ingestions/capture	
Source	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Zooplankton								
Length	17.80	0.0004	33.74	0.0004	35.23	0.0004	28.38	0.0004
Benthic invertebrates								
Length	33.67	0.0008	14.19	0.0008	4.97	0.0008	2.48	0.04
Density	6.57	0.0008	0.87	0.50	2.15	0.17	1.56	0.31
Fish								
Length	1.17	0.31	4.15	0.0008	0.79	0.72	2.51	0.06
Density	1.30	0.72	1.09	0.72	2.30	0.23	2.33	0.22

Note: Sums of squares are type III (SAS Institute Inc. 2001).

ciency exceeded 100% at some sizes because the walleye were able to capture several chironomids simultaneously. In contrast with zooplankton and benthic invertebrates, the probability of pursuit of fish prey did not vary significantly with either length or density (Table 2), as efficiency was generally high but variable (Fig. 3). Strike probabilities were influenced by walleye length but not fish density (Fig. 3). Capture efficiency was significantly influenced by fish density; capture efficiency was about 60% at low densities and decreased at high prey densities (Fig. 4). Capture efficiency was also much lower for fish than for both invertebrate prey types. Ingestion probabilities were highly variable across density and size (Fig. 4).

Functional responses

We determined the shape of the functional response curve for each prey type and walleye size class. Walleye exhibited a type II functional response to each prey type over a range of sizes (Fig. 2). The type II equation provided a reasonable fit to the data (R^2 mean = 0.72, range = 0.39–0.91) (Table 3). The only exception was the curve for the 30-mm fish trials that appears linear given that the estimated handling times were negligible. Estimates of the attack coefficient a did not vary among prey types ($F_{[2,28]} = 0.16$, $P = 0.85$) but did vary with walleye size ($F_{[1,28]} = 12.72$, $P = 0.006$). Attack coefficients on zooplankton and fish were fairly constant across sizes but decreased with length for the benthic invertebrates. Estimates of handling time T_h were influenced by prey type ($F_{[2,28]} = 6.53$, $P = 0.006$) and an interaction between prey type and walleye length ($F_{[2,28]} = 5.85$, $P = 0.009$). In general, handling time estimates for fish were greater than for both zooplankton and benthos, and handling time for benthos was noticeably greater than for zooplankton at small walleye sizes. Handling times decreased with walleye length for zooplankton. However, handling times for zooplankton were high for the 150-mm walleye, but this was a function of the walleye not eating the zooplankton rather than actual handling costs. Handling times decreased for benthic invertebrates with walleye size but were highly variable with size for fish prey.

Energy return

Energy return for each prey type generally increased with walleye size (Fig. 5). Energy return was highest on fish, intermediate on benthic invertebrates, and lowest on zooplankton for walleye greater than 30 mm. Small walleye (20–30 mm) had similar profitability on zooplankton and benthic invertebrates, but fish profitability was also higher for these two size classes. Energy return was only influenced by walleye length for zooplankton ($F_{[15,59]} = 341.55$, $P = 0.0001$) and fish ($F_{[15,59]} = 179.97$, $P = 0.0001$), but profitability of benthos was influenced by walleye length ($F_{[9,44]} = 762.59$, $P = 0.0001$), benthos density ($F_{[5,44]} = 8.94$, $P = 0.0001$), and their interaction ($F_{[1,44]} = 51.44$, $P = 0.0001$).

Walleye growth

Size ($F_{[4,60]} = 38.74$, $P = 0.0001$), prey type ($F_{[2,60]} = 70.42$, $P = 0.0001$), and their interaction ($F_{[8,60]} = 9.33$, $P = 0.001$) all affected growth of walleye. Growth rate was highest for fish prey across all size groups, but growth differed for walleye fed either zooplankton or benthic invertebrates depending on size (Fig. 5). Within each size class, prey type significantly influenced relative growth. At 20 mm, walleye had significantly greater growth on fish than on zooplankton and benthic invertebrates ($F_{[2,12]} = 28.18$, $P = 0.0001$) (Fig. 5). Growth was similar between zooplankton and benthic invertebrates at 20 mm. At 40 mm, growth was highest on fish, intermediate on benthic invertebrates, and lowest on zooplankton ($F_{[2,16]} = 5.67$, $P = 0.01$). At larger sizes (>40 mm), growth was negative on zooplankton, moderate on benthic invertebrates, and highest on fish prey and was significantly different among prey types ($P < 0.0001$) except

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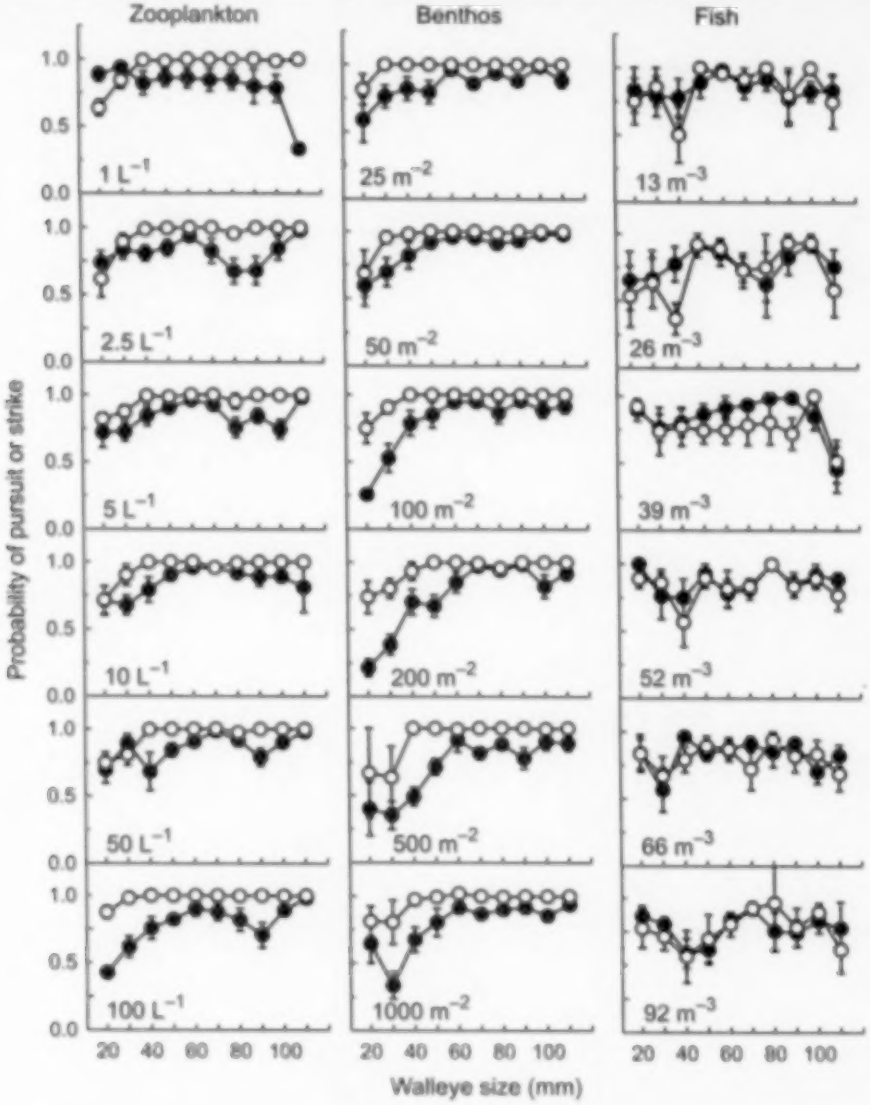
Fig. 3. Probability of pursuits after orientation (solid circles) and strikes after pursuit (open circles) on zooplankton, benthic invertebrates, and fish as a function of walleye (*Sander vitreus*) size for each prey density in feeding experiments. Values are means ± 1 SE.



for the 100-mm walleye where only the zooplankton and fish trials differed ($F_{[2,10]} = 8.10$, $P = 0.08$). Growth was not correlated with energy return for zooplankton (50·L^{-1}) ($r = -0.52$, $P = 0.28$) or benthic invertebrates (500·m^{-2}) ($r = 0.74$, $P = 0.08$) but was correlated with energy return for fish ($r = 0.83$, $P = 0.03$) (Fig. 5).

Discussion

Juvenile piscivores undergoing multiple diet shifts must pass several bottlenecks to survive through their first year of life. Initially, they are limited by mouth gape and swimming abilities (Gerritsen and Strickler 1977; Bremigan and Stein 1994). As predators grow, they become more proficient in capturing and handling prey and are able to include a wider variety of prey types in their diet (Mittelbach 1981; Olson 1996). Additionally, the ease with which a predator can successfully forage and availability of various prey types must be reconciled with growth consequences (Byström et al. 1998; Jonsson et al. 1999). To understand the intricacies of diet shifts of young piscivores, we must first understand how foraging abilities change with size and prey availability.

Young-of-the-year walleye foraging patterns were the result of changes in both walleye size and prey density. Previous field studies have demonstrated diet shifts as larval walleye consume zooplankton, switch to benthic macroinvertebrates as juveniles, and forage on fish in later juvenile and adult stages (Maloney and Johnson 1957; Priegel 1970). However, timing of these shifts is variable and likely the result of complex interactions among walleye

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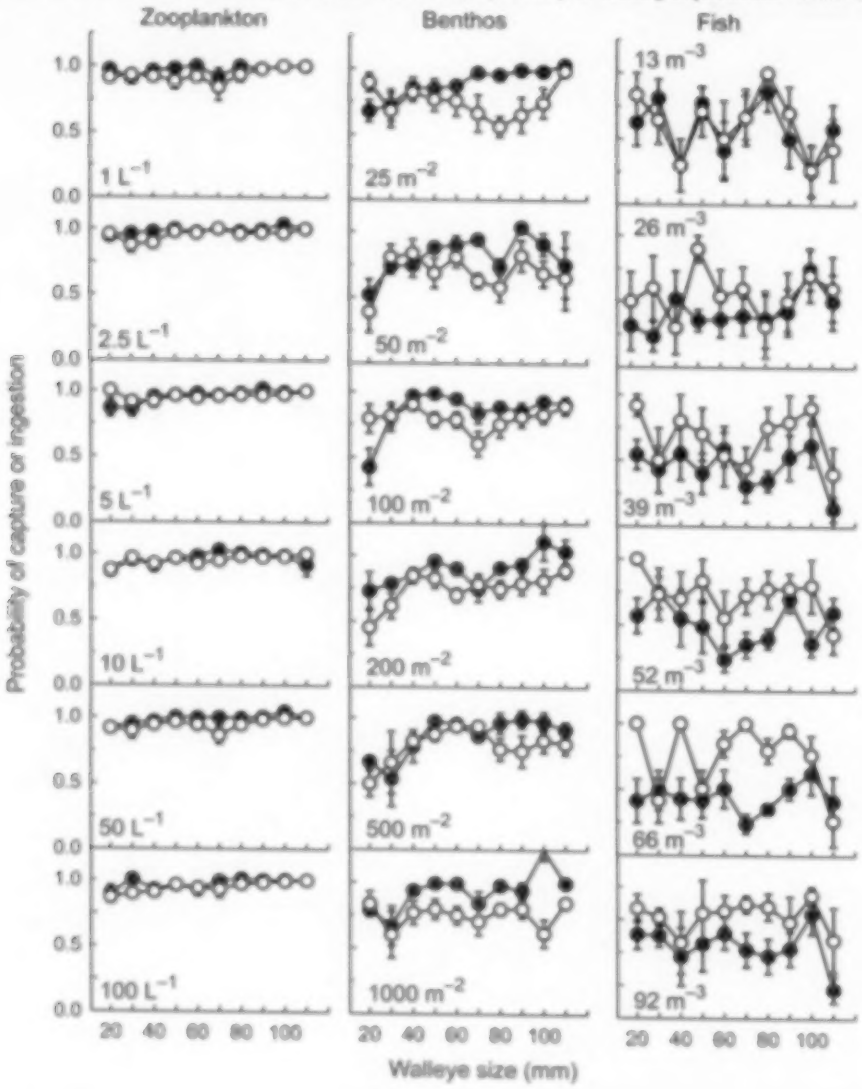
Fig. 4. Probability of captures after strike (solid circles) and ingestions after capture (open circles) on zooplankton, benthic invertebrates, and fish as a function of walleye (*Sander vitreus*) size for each prey density in feeding experiments. Values are means ± 1 SE.



size, prey type, and prey densities. We found ontogenetic changes in consumption of each prey type; as walleye size increased and foraging abilities improved, consumption increased. These ontogenetic patterns are supported by field observations; as walleye increase in size, the types and quantities of prey consumed increased (Houde 1967; Fox 1989). Small walleye (20 and 30 mm) ate few zooplankton relative to larger walleye; the largest size class (150 mm) ate almost no zooplankton. Small juveniles ate few chironomids relative to other walleye size classes. The effect of length on consumption of fish was less noticeable; walleye of all sizes easily consumed fish.

Prey availability also influenced consumption of each prey type. Walleye foraging was best described by type II functional responses, emphasizing the importance of prey densities. As density of each prey type increased, consumption increased until reaching an asymptote. Type II relationships have been found previously for fish feeding on zooplankton (Ringler and Brodowski 1983; Persson 1990; Miller et al. 1992) and benthic invertebrates (Ware 1972). Piscivores consume relatively fewer fish prey, however, and either type I or II responses could be appropriate depending on the importance of handling time (Breck 1993). Handling time can be defined in a variety of ways including the time from a successful attack until the initiation of a new search (Ranta and Nuutinen 1984; Hirvonen and Ranta 1996), from the first orientation towards a prey item to the completion of exaggerated gill movements (Gill and Hart 1996), and all of the time spent searching, capturing, and handling prey (Holling 1966). We used Holling's (1966) method where handling time is estimated by fitting a functional response to our experimental data (Juliano 1993). This method does not

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Table 3. Parameter estimates for Holling's type II functional response equation (±SE) as obtained from nonlinear regression.

Total length (mm)	Zooplankton			Benthic invertebrates			Fish		
	a (attacks·s^{-1})	T_h (s)	R^2	a (attacks·s^{-1})	T_h (s)	R^2	a (attacks·s^{-1})	T_h (s)	R^2
20	2.09×10^{-4} (1.34×10^{-4})	38.02 (5.88)	0.70	4.54×10^{-3} (3.85×10^{-2})	453.06 (108.89)	0.59	2.30×10^{-3} (1.80×10^{-3})	434.26 (115.06)	0.79
30	8.30×10^{-4} (7.52×10^{-4})	27.35 (4.27)	0.74	5.43×10^{-3} (2.32×10^{-2})	224.60 (47.24)	0.60	5.19×10^{-4} (2.73×10^{-4})	0 (224.42)	0.70
40	8.07×10^{-4} (6.11×10^{-4})	16.03 (2.50)	0.77	1.56×10^{-3} (1.13×10^{-3})	102.83 (16.26)	0.69	8.32×10^{-4} (7.38×10^{-4})	128.77 (255.34)	0.55
50	1.53×10^{-3} (1.03×10^{-3})	12.92 (1.40)	0.84	1.13×10^{-3} (3.33×10^{-4})	40.77 (3.64)	0.91	1.37×10^{-2} (4.41×10^{-2})	614.29 (118.15)	0.77
60	3.59×10^{-4} (1.75×10^{-4})	9.40 (1.38)	0.76	1.52×10^{-3} (5.17×10^{-4})	28.69 (2.84)	0.90	1.37×10^{-3} (1.08×10^{-3})	407.19 (161.99)	0.73
70	3.04×10^{-4} (1.82×10^{-4})	10.24 (1.76)	0.68	1.14×10^{-3} (7.14×10^{-4})	24.06 (5.52)	0.69	1.47×10^{-3} (7.96×10^{-4})	337.66 (103.38)	0.81
80	4.46×10^{-4} (2.68×10^{-4})	14.63 (2.32)	0.71	3.61×10^{-4} (1.91×10^{-4})	12.68 (1.98)	0.72	1.12×10^{-3} (5.26×10^{-4})	71.83 (110.09)	0.83
90	1.44×10^{-4} (1.11×10^{-4})	22.74 (5.79)	0.87	1.12×10^{-3} (6.00×10^{-4})	30.41 (4.53)	0.80	1.22×10^{-3} (7.81×10^{-4})	196.21 (137.98)	0.75
100	3.09×10^{-4} (3.26×10^{-4})	13.76 (4.40)	0.39	1.09×10^{-3} (4.08×10^{-4})	18.97 (2.70)	0.86	1.58×10^{-3} (9.24×10^{-4})	283.24 (105.11)	0.82
150	1.00×10^{-5} (2.00×10^{-5})	1217.00 (459.90)	0.50	4.87×10^{-4} (1.90×10^{-4})	21.01 (4.70)	0.83	4.35×10^{-4} (4.40×10^{-4})	223.91 (522.90)	0.40

Note: Regressions are based on number of zooplankton, benthic invertebrates, or fish consumed by different walleye (*Sander vitreus*) sizes in feeding experiments. Estimated parameters are the attack constant a and handling time T_h. Values of R^2 are given for each equation; all were significant ($P < 0.001$).

require empirically determining handling time directly in the laboratory, which can be logistically difficult for small prey types. Because type II models successfully fit our data and handling times were far greater than when feeding on zooplankton and benthic invertebrates, we conclude that handling times on fish by juvenile piscivores influence consumption (Breck 1993). In addition, handling times of small walleye (20 mm) were greater for chironomids than for zooplankton, potentially influencing prey profitability. Handling times of chironomids for larger walleye (>40 mm) decreased becoming similar to handling times for zooplankton. Small bluegill also experience high handling times on benthos relative to zooplankton, but unlike walleye, the handling times on benthos remain higher than for zooplankton as bluegills increase in size (Mittelbach 1981). Prey densities and corresponding handling times influence the number of each prey type consumed and appear to differ between major functional groups of fishes (e.g., invertivores and piscivores).

Although prey depletion and predator satiation can affect experimental foraging results, our trials were conducted over a short time period to reduce these effects, similar to or shorter than other laboratory feeding experiments (Stein et al. 1984; Czesny et al. 2002; Persson and Brönmark 2002). We did not replace prey during the trials, but there was no risk of prey being reduced to low levels given our visual observations, and the functional response curves fit to our data also account for depletion effects (Juliano 1993). Several models have been developed to account for satiation during a feeding bout (see review by Jeschke et al. 2002). Although Holling's disc equation does not implicitly define satiation effects, we felt that the model was the simplest and most appropriate method to describe our feeding relationships. It is unlikely that the small walleye became completely satiated during the trial when feeding on the relatively small zooplankton or benthic invertebrates, and satiation is not likely for piscivores (Essington et al. 2000).

Although walleye size and prey densities determine the total number consumed, differences among the three prey types influence foraging efficiencies. The decision to pursue zooplankton usually resulted in a successful capture, which is not surprising given that capture success of relatively small prey is often high (Drenner et al. 1978; Miller et al. 1988) and cladocerans have poor antipredatory skills (Szlauer 1965; Persson 1987; Mayer and Wahl 1997). Walleye were more likely to attack benthic invertebrates but had difficulty retaining captured chironomids. In addition, there was a low probability that small (20 and 30 mm) walleye would complete the predation sequence when feeding on benthic invertebrates. Unlike zooplankton and benthic invertebrates, fish prey have several antipredator tactics (evasion, spiny fins, and the ability to escape after capture) (Einfalt and Wahl 1997), resulting in relatively low capture success. Ontogenetic changes in consumption and inherent differences in the appearance, antipredatory skills, and habitat use of prey dictate the ability to exploit diverse prey and must develop early for walleye relative to other interspecific competitors.

Given differences in foraging abilities, we expected that profitability and growth of each prey type would change with walleye size. Walleye of all sizes consistently had greater energy return and grew larger when feeding on fish. Estimated profitability and growth on zooplankton and benthos were initially similar for small walleye (20–30 mm),

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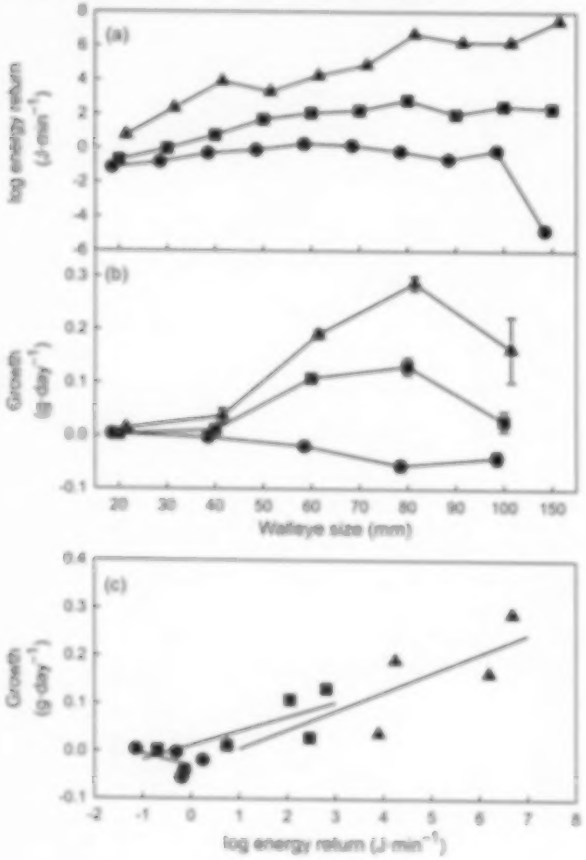
Fig. 5. (*a*) The log energy return for walleye (*Sander vitreus*) feeding on zooplankton (50·L^{-1}) (circles), benthic invertebrates (500·m^{-2}) (squares), and fish (66.1·m^{-3}) (triangles) in feeding experiments. Energy return was calculated as the energy consumed divided by handling time (eq. 2). (*b*) Growth of walleye of different sizes (20, 40, 60, 80, and 100 mm) fed ad libitum on either zooplankton (circles), benthic invertebrates (squares), or fish (triangles). Values are means ± 1 SE. (*c*) Correlation between energy return and growth of walleye fed zooplankton (circles), benthic invertebrates (squares), and fish (triangles).



and as walleye increased in size, energy gain from zooplankton remained low but increased on benthic invertebrates. Larger walleye (≥40 mm) had negative growth on zooplankton and moderate growth on benthic invertebrates. We found no relationship between energy return and growth for walleye fed zooplankton or benthic invertebrates ad libitum but a positive relationship when walleye were feeding on fish. In contrast, young-of-the-year Eurasian perch (*Perca fluviatilis*), which become piscivorous at a much larger size than walleye, exhibit a positive relationship between energy return and growth when fed both zooplankton and benthic invertebrates (Persson 1990). The negative relationship between energy return and growth on zooplankton indicates that zooplankton alone would be an insufficient diet resulting in poor growth and potentially starvation for walleye. Conversely, juvenile Eurasian perch can continue to exploit zooplankton without a negative impact on growth. Other piscivores such as largemouth bass (*Micropterus salmoides*) (Olson 1996) and pikeperch (*Stizostedion lucioperca*) (Buijse and Houthuijzen 1992) exhibit reduced growth in the field when prey fish are not available. The ontogenetic hierarchy of prey profitability influences growth of both piscivores and nonpiscivores, but because fish have greater caloric densities compared with zooplankton and benthos, their presence or absence is more likely to impact growth and survival. We expect that these relationships can be further refined in the future as handling times are directly measured in the laboratory and species-specific energy densities and digestibilities are calculated.

Our study suggests that young-of-the-year walleye diet shifts observed in the field (Walker and Applegate 1976; Mathias and Li 1982; Fox 1989) are the result of ontogenetic changes in foraging abilities, prey availability, and energetic consequences of prey selection. The initial shift from zooplanktivory to benthivory is the result of increased foraging abilities on benthic invertebrates and a decrease in energetic return on zooplankton. Shifts between these invertebrate groups have been attributed to energetic gain for other species (Mittelbach 1981; Persson and Greenberg 1990). Although fish are the most energetically profitable prey for all sizes of juvenile walleye, fish prey are typically not found in the diets of the small juveniles. We hypothesize that a variety of factors may lead to this discrepancy. Low densities or spatial isolation of fish prey of the appropriate sizes may explain any delays in switching to piscivory in the field; availability of fish has been linked to prey selection and growth for other percids (Buijse and Houthuijzen 1992; Stahl and Stein 1994). In addition, the high handling times combined with low capture success associated with fish prey relative to invertebrates could potentially increase predation risk for walleye (Sih 1980; Cerri and Fraser 1983; Nilsson and Brönmark 2000). Finally, our tank experiments lacked spatial complexity, which could potentially increase capture success of benthic invertebrates or fish. We expect that prey preferences of young-of-the-year walleye will also be influenced by the availability of alternative prey (T.L. Galarowicz and D.H. Wahl, unpublished data). Habitat complexity, predation risk, and competition also shape foraging patterns, and further investigation into the interaction of these factors is needed. Understanding both the invertebrate and fish diet shifts is crucial in predicting diet shifts and growth for young-of-the-year walleye and other juvenile piscivores.

Acknowledgements

We thank S. Stuewe, S. Krueger, and the staff of the Jake Wolf Memorial Fish Hatchery, Illinois Department of Natural Resources, for providing larval and juvenile walleye. We appreciate the collective efforts of the personnel of the Kaskaskia and Sam Parr biological stations for assistance with experiments. We are especially grateful to J. Adams, A. Daigle, L. Einfalt, K. Englund, K. Kirkham, and G. May with assistance in the laboratory. The comments of B. Graeb, R. Herendeen, the Aquatic Ecology Discussion Group, Kaskaskia Biological Station, and two anonymous reviewers improved this manuscript. M. Herbert contributed greatly to all aspects of this project. This work was funded

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by the Illinois Natural History Survey with additional support provided by the Department of Animal Biology, University of Illinois.

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Growth potential and host mortality of the parasitic phase of the sea lamprey (*Petromyzon marinus*) in Lake Superior

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Abstract: Landlocked Lake Superior sea lampreys (*Petromyzon marinus*) cause a significant but uncertain amount of mortality on host species. We used a sea lamprey bioenergetics model to examine the scope of host sizes vulnerable to death as a consequence of sea lamprey feeding and incorporated the bimodal lake-ward migration of parasitic sea lampreys. At their peak feeding rate and maximum size (P = 1.0, proportion of maximum consumption), spring migrants were capable of killing lean lake trout (*Salvelinus namaycush*) hosts ≤2.0 kg, which was larger than fall migrants (1.8 kg). Spring migrants feeding on Pacific salmon (coho (*Oncorhynchus kisutch*), Chinook (*Oncorhynchus tshawytscha*), and steelhead (*Oncorhynchus mykiss*)) killed hosts ≤2.0 kg, but fall migrants killed hosts as large as 2.8 kg. Although there is no direct empirical evidence, bioenergetics modeling suggests that it is plausible that some of the largest sea lampreys in Lake Superior spent more than one summer as parasites. Two-summer parasites readily attained sizes of sea-run adult anadromous sea lampreys and killed hosts from 3 to >5.5 kg in size. The maximum upper limit number of 2-kg hosts killed by two-summer parasites was nearly twice that of one-summer parasites.

Résumé : Les grandes lamproies marines (*Petromyzon marinus*) landlockées du lac Supérieur causent une mortalité significative, mais mal précisée, chez les espèces hôtes. Un modèle bioénergétique de la grande lamproie marine nous a permis de déterminer les tailles d'hôtes vulnérables à la prédation par les lamproies au point d'en mourir; le modèle incorpore la migration bimodale vers le lac des grandes lamproies marines parasites. À leur taux d'alimentation maximal et à leur taille maximale (P = 1,0, pourcentage de la consommation maximale), les migrateurs du printemps sont capables de tuer leurs hôtes, des touladis « minces » (*Salvelinus namaycush*) de ≤2,0 kg, ce qui est plus que les migrateurs d'automne (1,8 kg). Les migrateurs de printemps se nourrissant de saumons du Pacifique (coho *Oncorhynchus kisutsch*, quinnat *Oncorhynchus tshawytscha*) et de truites arc-en-ciel anadromes (*Oncorhynchus mykiss*) tuent des hôtes de ≤2,0 kg, alors que les migrateurs d'automne peuvent tuer des hôtes de taille aussi élevée que 2,8 kg. Même s'il n'existe pas de données empiriques d'appui, la modélisation bioénergétique indique qu'il est plausible que quelques-unes des grandes lamproies marines de plus grande taille au lac Supérieur passent plus d'un été comme parasites. Les parasites qui vivent deux étés atteignent facilement les tailles des grandes lamproies marines anadromes qui ont vécu en mer et peuvent tuer des hôtes dont la taille se situe entre 3 kg à >5,5 kg. La nombre supérieur maximal d'hôtes de 2 kg tués par les parasites qui vivent deux étés est presque deux fois celui des parasites d'un seul été.

[Traduit par la Rédaction]

Introduction

Interactions between predators and their prey have important feedbacks that govern the size, shape, morphology, behavior, and survival of each other (e.g., Alcock 2001). Host–parasite interactions are linked in a similar manner, and their interactions can take several forms. In the case of landlocked Great Lakes sea lampreys (*Petromyzon marinus*), they can take the form of an ectoparasite that inflicts nonlethal wounds during the course of feeding or a predator that removes enough host fluids to cause death directly or through secondary infection (Farmer and Beamish 1973; Farmer 1980; Swink 2003).

The successful invasion and colonization of the sea lamprey in Lake Superior contributed significantly to the decline of the historically dominant native top predator, the lean lake trout (*Salvelinus namaycush*) (Smith 1971; Lawrie and Rahrer 1972; Smith and Tibbles 1980). The sea lamprey is thought to have invaded Lake Superior from the lower Great Lakes in the late 1930s and was well established lake-wide about 10 years later (Smith and Tibbles 1980; Heinrich et al. 2003). Through chemical treatments of tributaries, mechanical/

Received 13 October 2004. Accepted 12 April 2005. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on 27 September 2005.
J18351

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 doi: 10.1139/F05-144 © 2005 NRC Canada

electrical barriers, and traps, sea lampreys in Lake Superior have been reduced and are controlled to about 10% of their historical abundance (Smith 1971; Smith and Tibbles 1980; Heinrich et al. 2003). Streams supporting the largest sea lamprey spawning runs were first treated in the western arm of Lake Superior beginning in the late 1950s and early 1960s, and the control program was expanded eastward into Wisconsin and Michigan tributaries. The control effort has widened and now encompasses each of the Great Lakes. Lake trout in Lake Superior have since recovered to near historical levels because of successful sea lamprey control efforts, stocking, and fishery regulations (Hansen et al. 1995; Bronte et al. 2003; Wilberg et al. 2003).

Although controlled to a fraction of their historical abundance, sea lampreys have increased in size since the control program began (described in detail in Jorgensen and Kitchell 2005). While their size has fluctuated, it increased significantly during the period 1961–2003. The mean weight of sea lampreys for the period 1999–2003 (mean = 200.9 g, n = 3607) was larger than that of the period 1961–1965 (mean = 173.5 g, n = 74 954; M. Henson, US Fish and Wildlife Service, Marquette Biological Station, 1924 Industrial Parkway, Marquette, MI 49855, USA, unpublished data). However, Great Lakes landlocked sea lampreys are considerably smaller than their anadromous conspecifics. Anadromous (sea-run) spawning sea lampreys can reach 1 kg, which is as much as four times the typical size of landlocked sea lampreys in the Great Lakes (Stier and Kynard 1986; Halliday 1991; Dempson and Porter 1993). If Great Lakes sea lampreys are conspecifics of anadromous sea lampreys, then Lake Superior sea lampreys' spawning size is a fraction of their potential for growth.

One reason cited for the difference in size between anadromous and landlocked sea lampreys is that anadromous sea lampreys are believed to spend more than 1 year at sea before reaching sexual maturity (Hardisty and Potter 1971; Beamish 1980). Investigators have hypothesized that landlocked Great Lakes sea lampreys spend only one summer as parasites (Applegate 1950; Johnson and Anderson 1980; Bergstedt and Swink 1995). Duration of the parasitic phase in the Great Lakes appears to be variable. Transformation to the parasitic phase and migration to the lake to feed occur over several months (Applegate 1950). Their migration timing is bimodal, one peak in the fall and another in the spring (Applegate and Brynildson 1951; Applegate 1961; Davis 1967). Host type, abundance, and preference as well as annual temperature cycles and host thermal preferenda are different for anadromous and landlocked sea lampreys, all of which could lead to variations in life history characteristics.

Whatever the causes of the differences in Lake Superior sea lamprey size and life history from their anadromous conspecifics, sea lamprey size has important consequences on host species. Larger sea lampreys are more fecund (Applegate 1950; Manion and Hanson 1980) and likely have higher survival to reproduction given the energy requirements of finding a tributary, swimming to suitable habitat in the tributary, and spawning, a period when no feeding occurs (Hardisty and Potter 1971; Beamish 1979). Food requirements are higher for larger sea lampreys, and feeding rates have direct effects on host survival (Farmer et al. 1975, 1977; Kitchell 1990). Significant blood removal weakens the host and can result in host death if the rate of blood removal exceeds the capacity of the host to replenish it (Farmer et al. 1975).

In this study, we address the following questions related to host death caused by blood removal by sea lampreys: (*i*) what is the size threshold of host survival under different sea lamprey feeding rates and size trajectories, (*ii*) how do host death threshold sizes differ for hosts occupying different thermal niches, and (*iii*) how do the size thresholds of host survival change owing to feeding by sea lampreys that spend two summers as parasites and that may grow to larger adult sizes?

Some aspects of these questions have been addressed by other workers through direct laboratory experimentation (Farmer and Beamish 1973; Farmer et al. 1975; Swink 2003) and (or) modeling (Lett et al. 1977; Kitchell and Breck 1980; Kitchell 1990). None has explicitly considered the bimodal sea lamprey migration timing and its implications for the length of the parasitic phase or extended projections of host size death thresholds caused by the feeding of two-summer parasites.

Materials and methods

Sea lamprey bioenergetics

To model growth and feeding rates of parasitic sea lampreys, we used a bioenergetics model developed by Kitchell and Breck (1980). Briefly, this model is based on a set of equations that balance energy in an organism:

$$(1) \qquad C = R + (F + U) + G$$

where consumption (C) equals metabolism (R) plus wastes (F, egestion, and U, excretion) and growth (G, somatic and (or) gonadal). We fit models to observed growth and thus were able to estimate blood consumption necessary to reach spawner weights. We parameterized the model as implemented in Fish Bioenergetics 3.0 (Hanson et al. 1997), with one difference (Table 1). Recent laboratory studies by Cochran et al. (2003) indicated that energy density for sea lampreys <119.5 g is described by the following equation:

$$(2) \qquad \text{Predator energy density} = 951.3 \text{ cal·g}^{-1} + 3.489W$$

where W is the mass (grams wet weight) of a sea lamprey. Once a sea lamprey reached the upper weight limit, predator energy density remained constant at 1368.6 cal·g wet weight^{-1} (Table 1) thereafter. This was incorporated into the model for sea lampreys that exceeded this weight cutoff by using the second equation set (Hanson et al. 1997; Table 1), which required setting the Beta2 parameter (the slope of the allometric function above the sea lamprey upper weight limit) to a very small number because a zero value produced spurious results. Prey energy density (host blood) was assumed to have the same caloric value for all host species (3202 J·g wet weight^{-1}; Farmer et al. 1975; Kitchell and Breck 1980).

By fitting the model to growth, we were able to estimate P, the proportion of maximum consumption. The value of P ranges from 0 to 1.0 (0%–100%), where 0 means no feeding and 1.0 signifies a fish feeding at its maximum rate given its

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Table 1. Inputs for sea lamprey (*Petromyzon marinus*) bioenergetics modeling (Kitchell and Breck 1980; Hanson et al. 1997; Cochran et al. 2003).

Parameter	Description/equation	Value
Consumption (C)	$C = C_{max}P_{max}f(T)$	—
$f(T)$	$V^{x}e^{x(1-V)}$	—
V	(CTM – T)/(CTM – CTO)	—
X	$(Z^2[1 + (1 + 40/Y)^{1/2}]^2)\cdot 400^{-1}$	—
Z	ln(CQ)(CTM – CTO)	—
Y	ln(CQ)(CTM – CTO + 2)	—
P_{max}	Proportion of maximum consumption	Fitted parameter
CA	Intercept of allometric mass function	0.3 $g\cdot g^{-1}\cdot day^{-1}$
CB	Slope of allometric mass function	–0.35
CQ	Coefficient of consumption	2.3
CTO	Temperature preferendum	18.0 °C
CTM	Upper incipient lethal temperature	25.0 °C
Respiration (R)	$R = RA\cdot W^{RB}\cdot f(T)\cdot ACT$	—
RA	Intercept of allometric mass function	0.00397 $g\cdot g^{-1}\cdot day^{-1}$
W	Fish mass (g)	—
RB	Slope of allometric mass function	–0.5
$f(T)$	$V^{x}e^{x(1-V)}$	—
V	(RTM – T)(RTM – RTO)$^{-1}$	—
X	$(Z^2[1 + (1 + 40/Y)^{1/2}]^2)\cdot 400^{-1}$	—
Z	ln(RQ)(RTM – RTO)	—
Y	ln(RQ)(RTM – RTO + 2)	—
RQ	Rate of increase over low water temperatures	2.1
RTO	Optimum temperature for respiration	25.0 °C
RTM	Maximum lethal temperature	30.0 °C
ACT	Activity multiplier	1.5
Egestation (F)	$FA\cdot C$	—
Excretion (U)	$UA(C - F)$	—
FA	Waste as constant proportion of consumption (C)	0.03
UA	Excretion as constant proportion of assimilated energy	0.15
Predator energy density (ED)	$\alpha + \beta W$ for $W \leq 119.6$ g	—
α	Intercept of allometric mass function	3982.0 $J\cdot g^{-1}$
β	Slope of allometric mass function	14.6
Mass cutoff	W at which function changes to constant value	119.6 g
ED for $W > 119.6$ g	—	5729.0 $J\cdot g^{-1}$
Prey energy density	—	3202.0 $J\cdot g^{-1}$

Note: Energy densities are expressed in wet mass.

size and temperature (Hanson et al. 1997). We began sea lamprey growth simulations at the same initial weight, 10 g (Applegate 1950; Kitchell 1990; Madenjian et al. 2003), for newly transformed parasites and fit the model to adult spawner weights that ranged from 100 to 400 g. This range of sizes captured the majority of spawning adult sizes observed in Lake Superior (M. Henson, US Fish and Wildlife Service, Marquette Biological Station, 1924 Industrial Parkway, Marquette, MI 49855, USA, unpublished data). In other simulations, we allowed the final end weights to exceed 400 g until P reached 1.0.

Proxies for sea lamprey thermal history were used in the model because actual thermal histories were not available. Sea lampreys likely spend much of their parasitic phase attached to hosts, whose thermal histories and (or) temperature preferenda are known. We used mean daily in situ temperatures recovered from Lotek thermal/depth archival tags planted in wild and hatchery-reared Lake Superior lean lake trout (Mattes and Madsen 2004). These temperatures ranged from 0.10 to 12.77 °C (Fig. 1). Pacific salmon (an amalgam of Chinook (*Oncorhynchus tshawytscha*) and coho (*Oncorhynchus kisutch*) salmon and steelhead (*Oncorhynchus mykiss*)) have been systematically stocked in large numbers and are important hosts for sea lampreys. Their thermal histories were based on mean near-surface water temperatures (McCormick 1996) measured in the 13-year period from 1980 to 1992 with modifications such that temperatures did not go below 3 °C or above 18 °C (Fig. 2) and thus were representative of a hypothetical Pacific salmon thermal history in Lake Superior (Christie and Regier 1988; Stewart and Ibarra 1991; Rand et al. 1993). For this study, we assumed that sea lampreys were always attached to and feeding on the host species indicated and therefore experienced the temperature of that host's thermal niche.

To incorporate the bimodality of newly transformed parasitic sea lampreys, we ran separate bioenergetics models for

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Fig. 1. Thermal history of lean lake trout (*Salvelinus namaycush*) derived from Lotek thermal archival tags implanted in Lake Superior wild and hatchery-reared fish in situ (Mattes and Madsen 2004). Mean temperatures (circles) are bounded by ±1 SD (shaded bars).

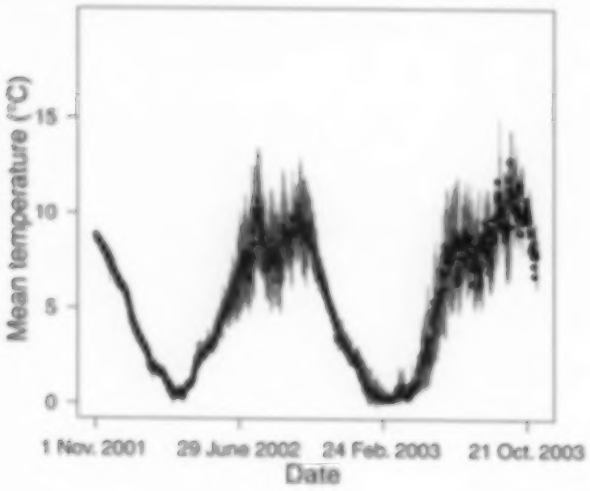
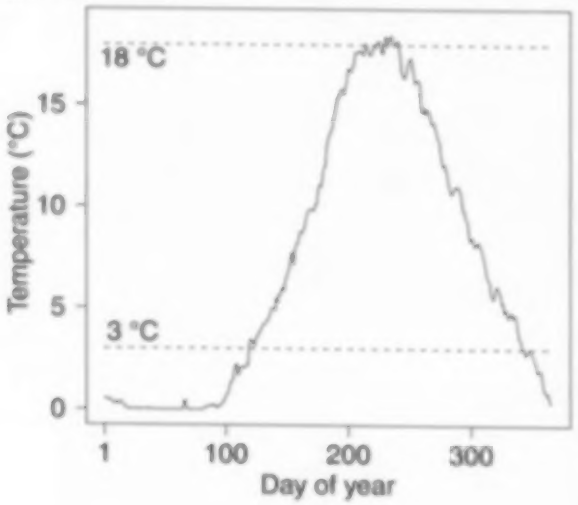


Fig. 2. Mean near-surface temperatures in Lake Superior (solid line) from the period 1980–1992 (McCormick 1996). Day 1 begins on 1 January. The broken lines indicate upper and lower cutoff values for the constructed Pacific salmon (*Oncorhynchus* spp.) thermal history.



spring and fall migrants. Migrants began feeding in either the fall (22 November) or the spring (1 April) and ceased feeding by spring of the next year (1 April), about 6 weeks before the beginning of the spawning run (Applegate 1950; Johnson and Anderson 1980). Thus, sea lampreys were parasitic for 12 and about 17 months, which included one summer as parasites (Applegate 1950, 1961; Johnson and Anderson 1980). We also ran simulations to model growth over two summers, the lower estimate of the hypothesized duration of the parasitic phase of anadromous sea lampreys, which corresponded to 24 and about 29 months as parasites.

Host size survival threshold

We based the calculation of host mortality on the rate of host blood removal (sea lamprey feeding rate) by finding a threshold of host survival size below which death occurred as a consequence of blood loss (Farmer et al. 1975; Kitchell 1990). Blood removal was calculated from sea lamprey feeding rates (which were assumed to feed every day) estimated on a daily time step. The lethal rate of daily blood removal was based on the experiments of Farmer et al. (1975, 1977), who estimated that a daily blood removal rate of 10% of the total amount of blood available was lethal to the host. Using this lethal blood removal rate, the host size death threshold is expressed by the following equation:

$$\text{(3)} \qquad \text{HSDT} = \text{FR}\frac{1000\ \text{g}}{4.04\ \text{g}}$$

where HSDT is the death threshold size of a host (expressed in g) and FR is the daily blood ration or sea lamprey feeding rate (grams per day) multiplied by the reciprocal of the percent blood by body weight of a 1000-g host, 4.04%. The percent blood of a fish by body weight was the mean value from studies of salmonid blood volume cited by Olson (1992) and from Bushnell et al. (1998). For this study, we illustrated the impact of a single feeding sea lamprey on a host, although multiple wounds are frequently observed on a host. One could adjust eq. 3 for multiple feeding bouts on the same host by multiplying the equation by the number of simultaneously feeding sea lampreys on a host.

Results

There was a strong seasonal pattern of increase in the sea lamprey feeding rate and hence size threshold of host survival. The strong peak, evident in all of the modeling scenarios, occurred in the fall when lake water temperatures inhabited by fishes were the highest. The start of the increase in host size death threshold occurred when waters began to warm, typically between early and mid-June, and the crest of the peak occurred in the fall, usually late September to mid-October. As discussed below, the contours and magnitudes of the host size death thresholds varied with the duration of parasitic phase and with host species.

Spring migrants, one-summer parasites

Spring migrants that spent 12 months as parasites and that utilized only lean lake trout as hosts were able to achieve a potential maximum weight of 248 g (proportion of maximum consumption, $P = 1.0$) (Fig. 3*a*). Mean weight for sea lampreys in 2003, 203 g, corresponded to a P value of 0.91 feeding only on lake trout for the entire 12-month parasitic phase (Fig. 3*a*). Hosts ≤1.2 kg were vulnerable to death during the seasonal feeding rate peak by sea lampreys reaching the maximum weight. The seasonal peak of the host size death threshold occurred at approximately day 200 (17 October).

Spring migrants that fed only on Pacific salmon had lower P values than those that fed only on lean lake trout at com-

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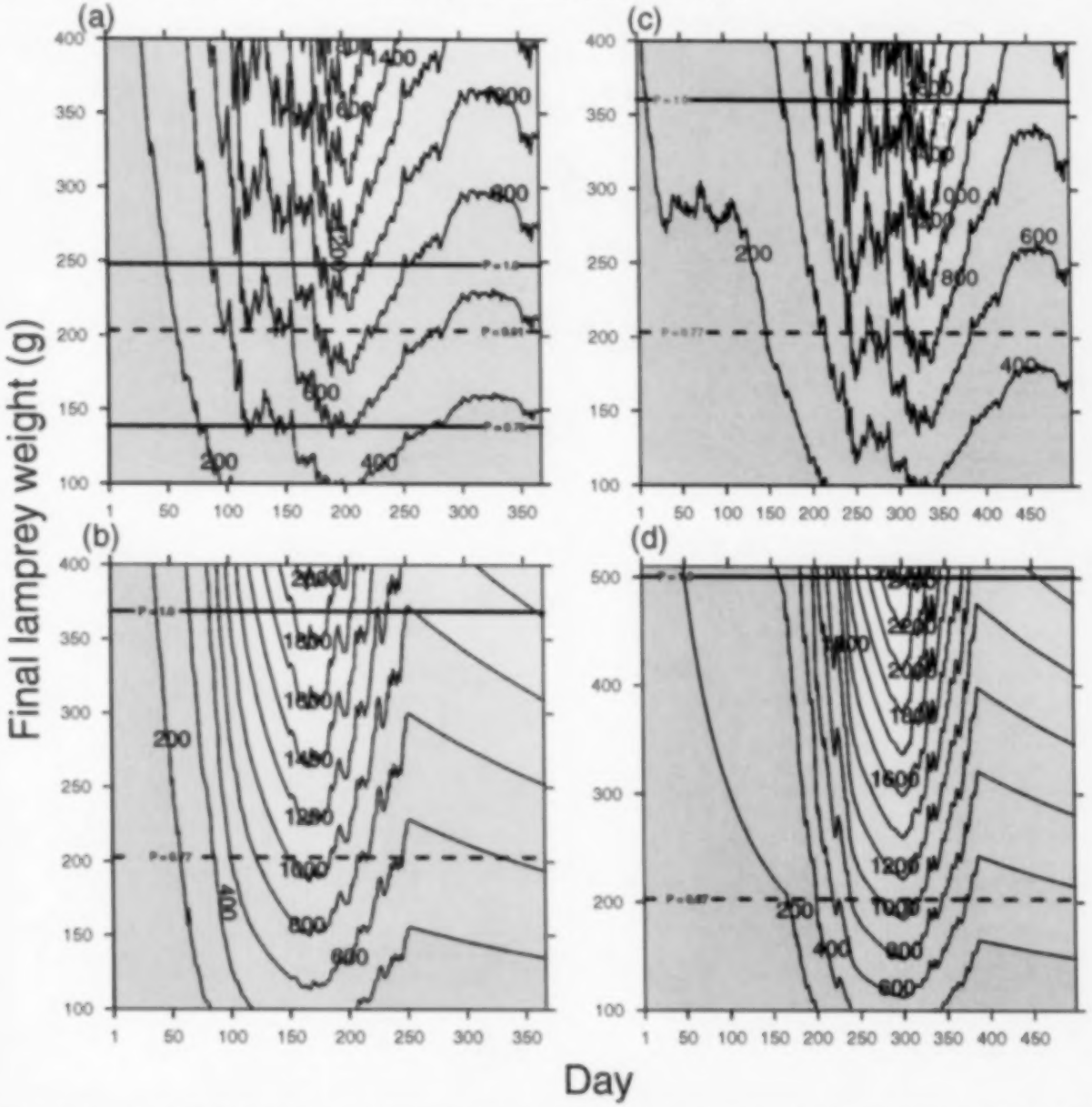
Fig. 3. Host size death threshold contour surfaces of parasitic sea lampreys (*Petromyzon marinus*) grown for one summer to different final adult spawning weights. Contour lines represent host death threshold sizes. Solid horizontal lines correspond to adult spawner weights where *P* (proportion of maximum consumption) values were equal to 1.0 (and 0.75 in *a*); broken horizontal lines indicate the fitted *P* values corresponding to final weights equal to the mean adult spawner weight in 2003. (*a* and *b*) Spring migrants spent 12 months as parasites and (*c* and *d*) fall migrants spent 17 months as parasites. (*a* and *c*) Contour death sizes of lean lake trout (*Salvelinus namaycush*; (*b* and *d*) contour death sizes of Pacific salmon (*Oncorhynchus* spp.).



parable sizes and were able to grow to a larger maximum size. These sea lampreys were able to achieve a maximum potential weight of 369 g (P = 1.0) (Fig. 3*b*), which was 49% larger than sea lampreys feeding only on lean lake trout. Sea lampreys that reached the 2003 mean spawner weight had a P value of 0.77. Hosts ≤1.8 kg were vulnerable to death by sea lampreys reaching the maximum size. Day 175 (22 September) was the seasonal peak of the host size death threshold.

Fall migrants, one-summer parasites

Sea lampreys that spent 17 months as parasites feeding only on lean lake trout were able to achieve a maximum potential weight of 360 g (P = 1.0) (Fig. 3*c*). This was slightly

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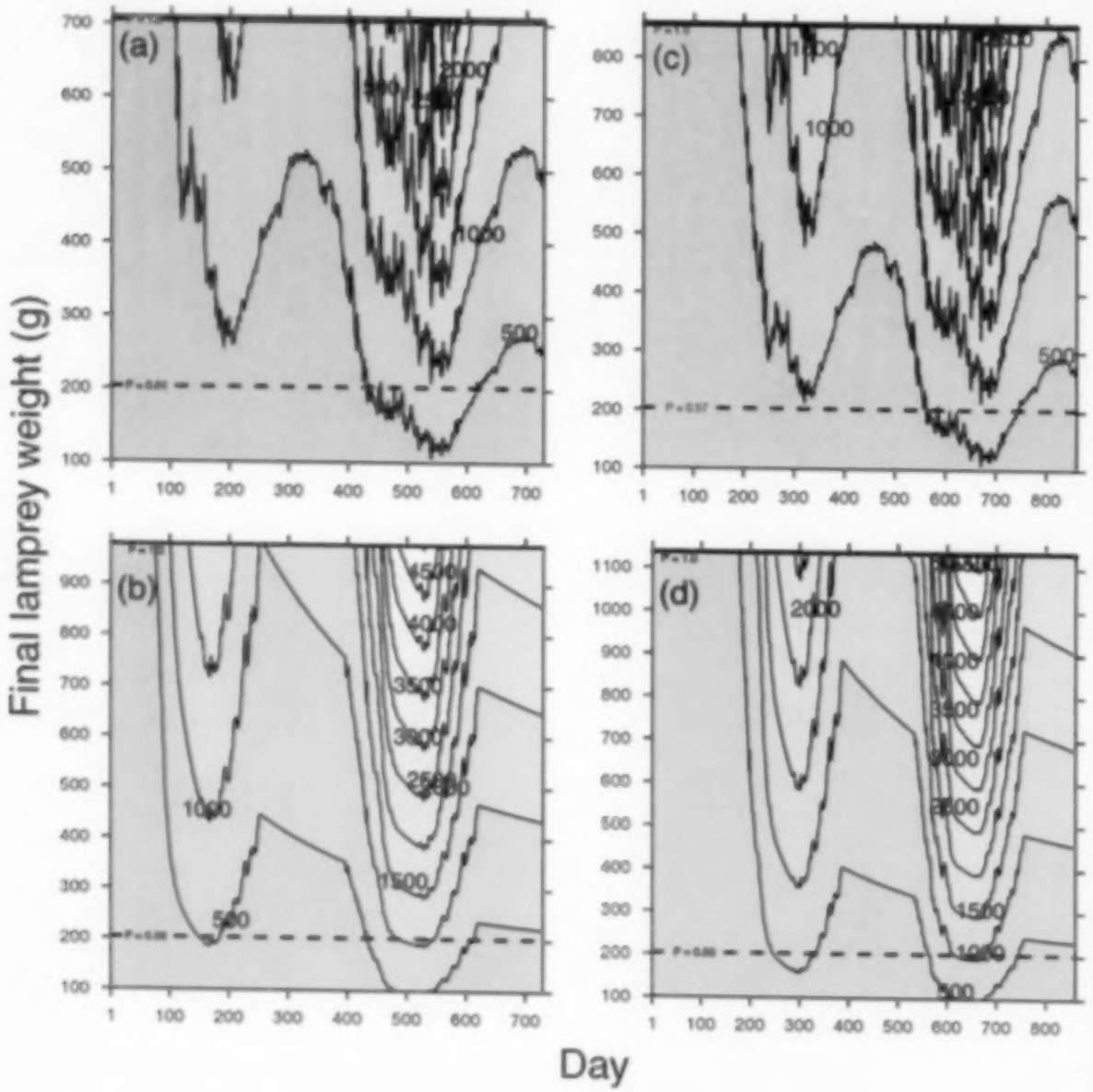
Fig. 4. Host size death threshold contour surfaces of parasitic sea lampreys (*Petromyzon marinus*) grown for two summers to different final adult spawning weights. Contour lines represent host death threshold sizes. Solid horizontal lines correspond to adult spawner weights where *P* (proportion of maximum consumption) values were equal to 1.0; broken horizontal lines indicate the fitted *P* values corresponding to final weights equal to the mean adult spawner weight in 2003. (*a* and *b*) Spring migrants spent 24 months as parasites and (*c* and *d*) fall migrants spent 29 months as parasites. (*a* and *c*) Contour death sizes of lean lake trout (*Salvelinus namaycush*); (*b* and *d*) contour death sizes of Pacific salmon (*Oncorhynchus* spp.).



less than spring migrants feeding only on Pacific salmon and 45% larger than spring migrants feeding only on lean lake trout hosts. The mean weight achieved by sea lampreys in 2003 required a *P* value of 0.77. Hosts ≤1.6 kg were subject to the threat of death during the seasonal feeding peak. Although these sea lampreys migrated about 5 months before the spring migrants feeding on lake trout, the peak of the size threshold of host survival was similar, day 325 (11 October).

Sea lampreys parasitizing only Pacific salmon hosts were able to achieve a maximum potential weight of 501 g (*P* = 1.0) (Fig. 3*d*), a 39% increase over the maximum weight achieved by fall migrants feeding only on lean lake trout hosts. Sea lampreys that reached the 2003 mean weight had a *P* value of 0.69. Even larger hosts (up to 2.6 kg) were vulnerable to death during the seasonal peak of the feeding rate. The peak of the host size death threshold was roughly day

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325 (11 October), which coincided with the peak date for sea lampreys feeding on lean lake trout.

Spring migrants, two-summer parasites

Spring migrants that spent two summers feeding only on lean lake trout (24 months as parasites) were able to achieve a maximum potential weight of 704 g (P = 1.0) (Fig. 4*a*). This was 184% larger than one-summer migrants utilizing the same host species. Sea lampreys required a P value of 0.61 to achieve the 2003 mean weight of 203 g, a 33% drop from one-summer spring migrants on the same host species. Hosts ≤3.5 kg were vulnerable to death at the peak of feeding by lampreys reaching the maximum weight. The peak of the host size death threshold occurred at day 550 (2 October).

Two-summer spring migrants that fed only on Pacific salmon achieved a substantially larger size than those feeding only on lean lake trout. For 24-month parasites, their maximum weight reached 980 g (P = 1.0) (Fig. 4*b*), a 39% increase from parasites feeding only on lean lake trout. This size increase was 166% larger than one-summer spring migrants feeding on the same host species. Sea lampreys that grew to the mean 2003 weight had a P value of 0.56. Hosts ≤5 kg were vulnerable to death during the peak feeding time by sea lampreys reaching the maximum size. The host size death threshold peaked at the same day as those feeding on lean lake trout, day 550 (2 October).

Fall migrants, two-summer parasites

Fall migrants that spent two summers as parasites (29 months) and that fed on lean lake trout grew to a maximum weight of 856 g (P = 1.0) (Fig. 4*c*), a 22% increase over spring migrants utilizing the same host species. The P value required to grow to the 2003 mean weight was 0.57. Hosts ≤4 kg were susceptible to death at the peak of feeding. Fall migrants that fed exclusively on Pacific salmon grew to the largest size of any of the simulations. The largest grew to a maximum potential weight of 1135 g (P = 1.0) (Fig. 4*d*), which was 33% larger than two-summer fall migrants feeding only on lean lake trout. These sea lampreys had the smallest P value, 0.53, of all of the other simulations necessary to achieve the 2003 mean weight. Hosts ≤5.5 kg, the largest of all of the simulations, were vulnerable to death. The day that the peaks of the host size death threshold occurred for both of these simulations was similar, at about day 675 (26 September).

Discussion

There were strong seasonal effects of host mortality that corresponded to a pulse in the sea lamprey feeding rate, and these seasonal effects were consistent with empirical observations of sea lamprey growth (Applegate 1950; Bergstedt and Swink 1995), host wounding rates (Spangler et al. 1980), and sea lamprey induced mortality patterns (Bergstedt and Schneider 1988). Lake water temperatures available to fishes are highest during the late summer – early fall period, and the sea lamprey feeding rate is a temperature-dependent function (Kitchell and Breck 1980; Hanson et al. 1997).

Maximum adult spawner weights achieved by sea lampreys depended on the duration of the parasitic phase, migration timing, and host type. Within the one- and two-summer parasitic time intervals, fall migrants were able to attain larger maximum spawning sizes than spring migrants, regardless of host type. Parker and Lennon (1956) found in laboratory studies that the total growth of fall lake-ward migrants was greater than that of spring migrants. Fall migrants, despite overwintering in the cold lake waters, spend a longer time as parasites and thus have more opportunities to feed than spring migrants. Host type was also important. A sea lamprey feeding on lean lake trout required a higher P value to reach the equivalent weight as a sea lamprey that fed on Pacific salmon over the same period. The thermal niches occupied by these hosts were the drivers of the differences in sea lamprey growth. Sea lampreys at higher temperatures had lower P values because estimates for P are derived from the proportion of maximum feeding multiplied by the reciprocal of the temperature dependence function (Hanson et al. 1997). Thus, P values are inversely related to temperature.

Sea lampreys that spent one summer as parasites had a high proportion of maximum consumption values compared with two-summer parasites and other bioenergetics modeling of fish growth. There may be several causes for the high P values. Spawning sea lampreys captured in 2003 included weights up to 400 g (M. Henson, US Fish and Wildlife Service, Marquette Biological Station, 1924 Industrial Parkway, Marquette, MI 49855, USA, unpublished data). The P value necessary for modeled growth to reach the observed growth for that year (203 g) was above 0.5 for all of the simulations and more frequently above 0.7. These P values were necessary for growth of the average size fish for that year. These values were high relative to bioenergetics models developed to model growth of other fishes (Stewart et al. 1983; Lantry and Stewart 1993; Negus 1995), which are rarely above 0.6. There are several possible explanations why sea lampreys grew larger than the mean weight in 2003: (*i*) they could have fed on host fishes with warmer thermal histories than were used in this study, (*ii*) their larger size could indicate that the bioenergetics model was incorrectly parameterized, (*iii*) some sea lampreys could spend more than 12–17 months (and more than one summer) as parasites, or (*iv*) some combination of these.

Temperature is an important factor for growth. Thermal histories of Pacific salmon were warmer than those of lean lake trout in these simulations. The other Great Lakes are examples of lake conditions, compared with Lake Superior, where warmer waters and potential hosts that occupy these waters are abundant. Sizes of sea lampreys in other Great Lakes are routinely 50–100 g larger than those in Lake Superior (e.g., Great Lakes Fishery Commission Annual Reports, www.glfc.org), which is not surprising, since it is the coldest of the five Great Lakes (see Assel et al. 1994). Sea lamprey temperature preferences (McCauley et al. 1977) appear to be closer to those of Pacific salmon than to those of lean lake trout (Wismar and Christie 1987; Bergstedt et al. 2003; Mattes and Madsen 2004), which should impart a lower P value to sea lampreys feeding on warmer-water hosts.

Hosts that occupy warmer waters when they are available provide an opportunity for sea lampreys to grow to larger sizes than cold-water hosts. The potential for differential growth is illustrated by the lower P values of sea lampreys that parasitized Pacific salmon compared with the same-

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sized sea lampreys that parasitized lake trout. Although size selective, sea lamprey wounds have been observed on most of the larger fishes found in the Great Lakes (Parker and Lennon 1956; Farmer and Beamish 1973; Johnson and Anderson 1980). Recent investigations of the Lake Superior food web suggested that sea lampreys may not be feeding exclusively on hosts at the highest trophic position and that lower trophic position species such as suckers (family Castostomidae) and lake whitefish (*Coregonus clupeaformis*) may be important hosts for sea lampreys (Harvey and Kitchell 2000; Harvey 2001; C. Harvey, NOAA Fisheries, 2725 Montlake Boulevard E., Seattle, WA 98112, USA, unpublished data). If these hosts are utilized to any extent by sea lampreys, then they would increase sea lamprey growth rates, and given their smaller size, these hosts would suffer the highest mortality rates.

The high P values could be the result of incorrect model parameterization or assumptions. Since the sea lamprey bioenergetics model was developed (Kitchell and Breck 1980), new information about predator energy density and host thermal histories has become available (Cochran et al. 2003; Mattes and Madsen 2004). Using model refinements in conjunction with laboratory studies of blood consumption and growth similar to Farmer et al. (1975) would further improve model inputs. Values of P determined from the original model calibration (Kitchell and Breck 1980), based on the laboratory studies of Farmer et al. (1975), ranged from 0.45 to 0.65, the highest of which corresponded to sea lampreys feeding on lean lake trout in Lake Superior. Previous growth simulations were run for 12 months only and started on 1 January (Kitchell and Breck 1980; Madenjian et al. 2003) and did not take into account the bimodal migration timing of transformed sea lampreys.

For comparison, we used Kitchell and Breck's (1980) estimated thermal history for lean lake trout hosts to compare the percent change in P values and total consumption with in situ lean lake trout temperatures for growth of the mean 2003 sea lamprey (203 g):

$$\frac{\text{This study} - \text{Kitchell \& Breck (1980)}}{\text{Kitchell \& Breck (1980)}} \times 100$$

We parameterized the model as per this study, and growth was modeled for 12 and 17 months. The P values increased by 21% and 16% for 12- and 17-month parasites, respectively, from the estimated to the in situ thermal history, while total blood consumption decreased 12% for parasites of the same growth period (Table 2). Thus, the previous analysis may have underestimated the proportion of maximum consumption and overestimated total blood consumption on lean lake trout. The discrepancies between the P values in this study and those of the earlier study suggest that perhaps the model parameters and assumptions about migration timing may need reevaluation with more laboratory and field study.

Consumption and growth outputs from bioenergetics models are sensitive to changes in model parameters and data inputs. The results that we obtained here are vulnerable to the same set of limitations of other such models (e.g., Ney 1993). As illustrated above, the model is highly sensitive to temperature. Several studies have shown that the activity multiplier can be highly variable and also can have a strong effect on model estimates of growth and consumption (Boisclair and Leggett 1989; Boisclair and Sirois 1993). The activity multiplier in this study was assumed to be 1.5 (Kitchell and Breck 1980), which is based on the laboratory experiments of Farmer et al. (1977). The activity multiplier's in situ distribution in the Lake Superior sea lamprey population is probably highly variable, but it was probably not responsible for the discrepancy between observed growth and model predictions at maximum P because it was shown to minimally increase the potential for error in consumption estimates (Kitchell and Breck 1980). Although temperature, activity metabolism, and other parameters vary across individuals, we assumed that the parameters and input data represented the average sea lamprey and how changes in migration timing, host choices, and length of the parasitic phase could affect consumption and growth. Recent advances in the model's parameters (predator energy density; Cochran et al. 2003) and data inputs (in situ lake trout thermal histories; Mattes and Madsen 2004) have presumably improved the model's performance in estimating sea lamprey blood consumption (Ney 1993; Hansen et al. 1993) and therefore host death size thresholds (Kitchell 1990).

Table 2. Comparison of bioenergetics model outputs between the estimated Kitchell and Breck (1980) (KB) thermal history for lean lake trout (*Salvelinus namaycush*) with in situ temperatures (Mattes and Madsen 2004).

Thermal history	Life span (months)	P	Total blood consumption (g)
KB	12	0.75	876.5
In situ	12	0.91	772.4
KB	17	0.67	974.4
In situ	17	0.77	862.5

Note: Model outputs are based on growth of mean size sea lamprey (*Petromyzon marinus*), 203 g, in 2003.

A recent individual-based modeling approach suggested setting the P value equal to 1.0 for sea lampreys <160 g, with an exponential decline thereafter (Madenjian et al. 2003). Optimal foraging theory suggests that animals strive to maximize food benefits while reducing the cost to obtain those benefits (e.g., Townsend and Winfield 1985). If sea lampreys follow this strategy, they should maximize host attachment time and minimize the search for new hosts (Cook and Cockrell 1978; Sih 1980; Cochran and Kitchell 1989). Sea lampreys have been observed attached to hosts without initiating feeding and remained attached to the same host for long periods without inducing host mortality (Parker and Lennon 1956; Cochran 1984), which may suggest that they are balancing the costs associated with finding a new host with the benefits of remaining attached to a host with some capacity to replenish its blood supply. The lower the sea lamprey to host weight ratio, the longer a sea lamprey can remain attached and feed (Swink 2003 and references therein). Thus, there may be some evidence for adjusting the P value as Madenjian et al. (2003) suggested for smaller sea lampreys, but further laboratory validation similar to Farmer et al. (1975) is needed.

In any case, this analysis suggests that some fraction of Lake Superior sea lampreys have a longer parasitic phase

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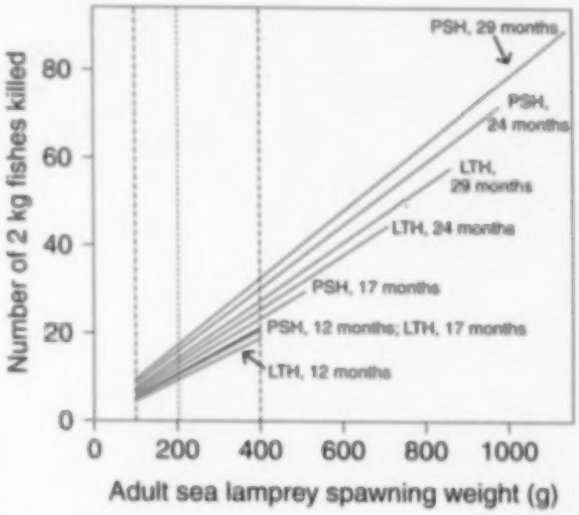
than previously hypothesized. Although there is no direct evidence to support this (Bergstedt and Swink 1995), bioenergetics modeling suggests that it is plausible from a growth standpoint. The proportion of maximum consumption decreased the longer a sea lamprey spent as a parasite and the warmer the host species' thermal history. The P values decreased substantially as simulations increased in duration from one summer to two. The lowest P values are more consistent with other bioenergetics model simulations of the growth of other Great Lakes fishes (Stewart et al. 1983; Lantry and Stewart 1993; Rudstam et al. 1995). Anadromous sea lampreys are hypothesized to have a parasitic phase about two times the length of Great Lakes sea lamprey parasites, and they grow to about three times (or more) their size (Beamish 1980; Yergeau 1983; Stier and Kynard 1986). The reasons for this are not known, but perhaps the energy requirements necessary for anadromous sea lamprey spawning in coastal rivers require a higher buildup of biomass (Beamish 1979, 1980). Perhaps there are size-selective predatory pressures (e.g., Brooks and Dodson 1965) on anadromous sea lampreys in their oceanic parasitic phase that may cause an upward shift in the adult size distribution. Certainly, the thermal histories experienced by sea lampreys in the Atlantic Ocean are probably quite different from those of landlocked sea lampreys, and sea lampreys in the Atlantic Ocean can attack very large hosts with enormous blood pools. Regardless, anadromous sea lampreys reveal the potential for growth of their landlocked conspecifics. Since their invasion into Lake Superior, landlocked sea lamprey growth has not reached its potential but a small fraction approaches it. Parker and Lennon (1956) reported that three out of 22 sea lampreys grown in laboratory studies, although reaching sizes comparable with those of others that spawned, failed to reach sexual maturity after the usual parasitic-phase life cycle period. Two other studies documented captures of large, presumptively spawning weight sea lampreys in the Great Lakes after the traditional spawning period was over (Moore et al. 1974; Bergstedt and Swink 1995). Setting P = 1.0 for one-summer parasites feeding exclusively on Pacific salmon still does not account for the largest sea lampreys observed. The overall high P values for one-summer parasites suggest that large sea lampreys either are extremely aggressive feeders or spend more time as parasites to reach observed growth. Those observations, together with the results presented in this study, suggest that some fraction of sea lampreys have a longer parasitic phase than previously hypothesized.

The damage to hosts caused by sea lampreys is difficult to measure. Direct in situ host mortality as a consequence of parasitic feeding is elusive. The total pool of blood consumed by sea lampreys likely represents a considerable amount of fish killed. Recent estimates of sea lamprey induced mortality on lean lake trout, based on wounding rates, suggest that the number of lean lake trout killed by sea lampreys is increasing (M. Ebener, Chippewa Ottawa Resource Authority, 179 West Three Mile Road, Sault Ste. Marie, MI 49783, USA, unpublished data). A hypothesized maximum upper limit number of fishes killed by a sea lamprey based on the results of this study is depicted in Fig. 5 using total blood consumption divided by the amount of blood in a 2-kg fish. These values are speculative estimates of the maximum possible number of fishes killed directly by feeding sea lampreys of differing growth trajectories, duration of the parasitic phase, and host type. Actual death rates depend on the hosts' capacities to replenish the blood removed. Sizes of spawning Lake Superior sea lampreys are largely <400 g, which suggests that up to 20 host fishes 2 kg in size could be killed by sea lampreys that spend one summer as parasites. A 203-g sea lamprey (the mean weight in 2003) feeding on lean lake trout (12–17 months as a parasite) and Pacific salmon (12 months) hosts could have potentially killed a maximum of 9–12 fishes. The number of fishes killed by a sea lamprey of the same size increases for parasites that switched from lean lake trout to Pacific salmon or a 17-month parasite feeding on Pacific salmon. What is most striking is the increase in the maximum number of fishes that can be killed by large parasites spending more than one summer as parasites. Even at final weights between 100 and 400 g, the number of fishes killed is about three times the maximum upper limit killed by one-summer parasites (Fig. 5).

Fig. 5. Maximum upper limit number of 2-kg fishes killed as a result of sea lamprey (*Petromyzon marinus*) feeding on indicated hosts. Hosts are denoted by PSH (Pacific salmon hosts, *Oncorhynchus* spp.) and LTH (lake trout hosts, *Salvelinus namaycush*). The numbers following the hosts indicate the time in months that sea lampreys spent as parasites. Lines extend to maximum adult spawning weights reached by sea lampreys with P = 1.0 or 400 g, whichever was higher. Broken vertical lines at 100 and 400 g indicate the size range of the majority of adult spawner sizes in Lake Superior, and the dotted vertical line shows the mean sea lamprey weight for 2003 (203 g; M. Henson, US Fish and Wildlife Service, Marquette Biological Station, 1924 Industrial Parkway, Marquette, MI 49855, USA, unpublished data).



The research presented here suggests revisiting some of the sea lamprey bioenergetics parameters and assumptions. Further studies with thermal/depth archival tags implanted into sea lampreys, in conjunction with the stable isotope analysis of the Lake Superior food web (C. Harvey, NOAA Fisheries, 2725 Montlake Boulevard E., Seattle, WA 98112,

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USA, personal communication) and sea lamprey statolith aging studies (M. Steeves, Department of Fisheries and Oceans, 1 Canal Drive, Sault Ste. Marie, ON P6A 6W4, Canada, personal communication), will reveal more information about the length of the parasitic phase, host selection, and their potential for growth in Lake Superior.

Acknowledgements

We are greatly indebted to B. Mattes (GLIFWC) and R. Bergstedt (USGS) for lake trout thermal history data. We thank M. Ebener (CORA), G. Christie (GLFC), R. Bergstedt, and B. Swink (USGS) for valuable discussions. We kindly thank M. Fodale, M. Henson, and R. Kahl (US Fish and Wildlife Service, Marquette Biological Station, 1924 Industrial Parkway, Marquette, MI 49855, USA) for sea lamprey size data. D. Sarker provided helpful instructions with the R's lattice package. C. Harvey, I. Kaplan, L. Schubert, and an anonymous reviewer provided valuable comments on an earlier version of this article. This research was supported in part by the US Department of Commerce, National Oceanic and Atmospheric Administration, Sea Grant College Program Award No. NA16RG2257, the State of Wisconsin, and an Anna Grant Birge Memorial Award.

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Sea lamprey (*Petromyzon marinus*) size trends in a salmonid stocking context in Lake Superior

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Abstract: Fish community objectives for Lake Superior call for restoration such that it resembles its historical species composition, to the extent possible, yet allow for supplementation of naturalized Pacific salmonids (*Oncorhynchus* spp.). To achieve these goals, managers strive to control the sea lamprey (*Petromyzon marinus*) to levels that cause insignificant (<5%) mortality to host species. While control efforts have been successful, sea lamprey size has increased during the control period. We analyzed long-term sea lamprey size trends and found a significant increase from 1961 to 2003 ($F = 36.76$, $p < 0.001$, $R^2 = 0.473$). A local regression revealed two significant size increase periods. We used Bayesian model averaging to find the relationship between sea lamprey size and the stocking of salmonids (lean lake trout (*Salvelinus namaycush*) and Pacific salmon). Bayesian model averaging identified 91 models, and several regressors were common features in many of the models. Sea lamprey weight was related to stocked lake trout lagged 3, 9, 11, and 13 years, and stocked Pacific salmon lagged 4 years. If sea lampreys can achieve larger sizes attached to Pacific salmonid hosts, and thus inflict more damage, there may be a trade-off for managers in achieving the fish community objectives for Lake Superior.

Résumé : Les objectifs définis pour la communauté de poissons du lac Supérieur incluent une restauration d'une composition des espèces qui ressemblerait à celle du passé, mais qui accommoderait l'addition des salmonidés du Pacifique (*Oncorhynchus* spp.) naturalisés. Pour atteindre ces buts, les gestionnaires tentent de maintenir les populations de grandes lamproies marines (*Petromyzon marinus*) à des densités qui ne causent qu'une mortalité négligeable (<5 %) aux espèces-hôtes. Bien que les programmes de contrôle aient eu du succès, la taille des grandes lamproies marines a augmenté durant leur application. Nous avons analysé les tendances des tailles des grandes lamproies marines et avons découvert un accroissement significatif de 1961 à 2003 ($F = 36{,}76$, $p < 0{,}001$, $R^2 = 0{,}473$). Une analyse de régression locale révèle l'existence de deux périodes d'accroissement significatif de la taille. Nous avons utilisé une combinaison bayésienne des modèles alternatifs (MBA) pour établir la relation entre la taille des grandes lamproies marines et les empoissonnements des salmonidés (touladis « minces », *Salvelinus namaycush*, et saumons du Pacifique). La procédure MBA a identifié 91 modèles qui possèdent plusieurs variables prédictives en commun. La masse des grandes lamproies marines est en relation avec les empoissonnements de touladis avec un décalage de 3, 9, 11 et 13 ans et avec ceux des saumons du Pacifique avec un décalage de 4 ans. Si les grandes lamproies marines peuvent atteindre des tailles plus élevées lorsqu'elles se fixent à des saumons du Pacifique comme hôtes et ainsi causer plus de dommages, il peut exister un compromis pour les gestionnaires à atteindre les objectifs qu'ils se sont fixés pour la communauté de poissons au lac Supérieur.

[Traduit par la Rédaction]

Introduction

The fish community of Lake Superior has changed dramatically in response to fishery activities and the invasion of exotic species (Christie 1974; Bogue 2000). The historical abundance of the top predator in Lake Superior, lean lake trout (*Salvelinus namaycush*), was depleted by fishery exploitation and the successful colonization of the sea lamprey (*Petromyzon marinus*) (Smith 1971; Smith and Tibbles 1980). By the late 1950s, lean lake trout had been extirpated from all of the Great Lakes except the western arm of Lake Superior (Christie 1974; Lawrie 1978). In response to these and similar patterns of the decline of fishes in the other Great Lakes, a binational program to control sea lampreys was implemented in the 1950s (Heinrich et al. 2003). US and Canadian resource managers erected mechanical and electrical barriers on selected tributaries to prevent sea lampreys from reaching spawning grounds. At these sites, managers recorded biological characteristics, such as weight and sex.

Biological characteristics of sea lampreys have been used by investigators to infer information about lamprey and host abundance. Heinrich et al. (1980) attempted to couple the

Received 13 October 2004. Accepted 12 April 2005. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on 27 September 2005.
J18352

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 doi: 10.1139/F05-145 © 2005 NRC Canada

relative abundance of sea lampreys with weight over a long-term period. For each year in the period 1958–1978, they fitted length–weight models, and from this, they computed the weight at length corresponding to the average length of sea lampreys in the Great Lakes. Heinrich et al. (1980) found a significant positive correlation between annual average sea lamprey weight with lake trout abundance the previous year in Wisconsin waters of Lake Superior. They also found a negative correlation between sea lamprey size and abundance. Thus, their analyses suggested some density dependence among sea lampreys for host resources: as sea lamprey abundance decreased, the size of sea lampreys increased, and when more hosts were available, sea lamprey size increased. They concluded that three interrelated factors influenced sea lamprey size: (*i*) sea lamprey abundance, (*ii*) the effectiveness of the sea lamprey control program, and (*iii*) the availability of preferred host species.

Most recently, Houston and Kelso (1991) assessed the relationship of sea lamprey biological characteristics and salmonid abundance. First, they described changes in biological characteristics over time. They reported that sea lamprey weight decreased through the 1950s, increased during the period 1962–1972, stabilized during the period 1973–1978, and increased during the period 1979–1987. They regressed two biological characteristics (weight and percent males in the population) on three predictor variables separately: salmonid stocking (an aggregation of eight species), commercial salmonid catch, and sea lamprey relative abundance. For Lake Superior, Houston and Kelso (1991) found that both sea lamprey weight and the percentage of males in the population were significantly positively related to salmonid catch but not to salmonid stocking. However, it is not clear how the aggregation of stocked salmonids was time-lagged to account for their growth necessary to become suitable hosts.

We had two objectives for this study. First, we analyzed long-term trends of sea lamprey weight in Lake Superior, which included data collected subsequent to the last trend analysis. Second, we employed Bayesian model averaging (BMA) to select a subset of models that related sea lamprey weight to the stocking of lean lake trout and Pacific salmon (*Oncorhynchus* spp.) in Lake Superior. Our research questions were (*i*) what are the long-term trends in sea lamprey size, (*ii*) how is salmonid stocking related to sea lamprey size, and (*iii*) what size forecasts can be made based on past stocking efforts?

Materials and methods

Data

The US Fish and Wildlife Service maintains a database that contains biological characteristics of adult spawning sea lampreys caught by electrical and mechanical barriers as well as portable traps in US tributaries to Lake Superior. Since the early 1950s, personnel have annually recorded weight of a subsample of sea lampreys caught. Subsample sizes of measured fish were larger in earlier years (1000s) than in later years (100s). Although not every tributary was sampled each year, the 25 tributaries in the data set span the length of the southern shore of Lake Superior from western Wisconsin to the eastern edge of Michigan's Upper Peninsula. Sea lampreys can move long distances during their parasitic phase (Smith and Elliott 1952; Moore et al. 1974; Bergstedt et al. 2003) and there is no compelling evidence suggesting that they home to their natal streams (Applegate and Smith 1951; Bergstedt and Seelye 1995). Therefore, we assumed that sea lampreys caught in these streams likely represent lake-wide independent random samples of spawning adults.

Sea lamprey collection methods included barriers (electrical and mechanical; from the beginning of the control program to 1975 and 1977–1979) and portable traps (1975 to the present). The different capture methods may have had different size selectivities, but the data do not contain enough overlapping years in which both barriers and portable traps were used to standardize size between collection methods. Thus, we ignored the method of collection, and combined measurements to compute mean sizes if multiple capture methods were used in the same year.

We obtained fish stocking records for Lake Superior from the Great Lakes Fishery Commission (GLFC; http://www.glfc.org/fishstocking/). Records of fishes stocked in Lake Superior begin in 1950 and continue through 2002. Lean lake trout stocking began in 1950, and systematic stocking of Pacific salmon began in 1967. We define Pacific salmon here as an amalgam of Chinook (*Oncorhynchus tshawytscha*) and coho salmon (*Oncorhynchus kisutch*) and steelhead (*Oncorhynchus mykiss*). We chose to use stocking rates, since historical long-term lake-wide abundance estimates of these fishes are not available.

Modeling

To accomplish the first goal of analyzing size trends of sea lampreys over time, we used two different procedures. We used weight as a measure of size (after Houston and Kelso 1991), and since there was no statistical difference in weight between males and females ($p < 0.05$), we pooled data for both sexes. For size trend analysis, we limited the data to the period 1961–2003, the period after nearly full sea lamprey control efforts were in place (Heinrich et al. 2003). First, we fitted a simple least squares linear regression model of weight over time to describe the general trend. Second, we fitted a loess curve, a locally distance-weighted least squares polynomial regression (Venables and Ripley 2002), to capture both short- and long-term weight trends. We set the span (α, the percentage of data points used in the localized fitting, here equal to 0.6) such that the loess curve captured decadal trends without overfitting the data.

For the second goal of relating salmonid stocking to sea lamprey size, we used BMA to select a subset of multiple linear regression models that related sea lamprey weight to salmonid stocking. The framework is based on the idea that sea lamprey weight today is a function of salmonids stocked some number of years before present. In its general form, this is represented by

$$(1) \qquad W_t = \beta_0 + \sum_{i=1}^{m} \beta_{1i}\, \mathrm{LT}_{t^{\circ}} + \sum_{j=1}^{n} \beta_{2j}\, \mathrm{PS}_{t^{\circ}} + \varepsilon$$

where W_t represents mean lamprey weight in year t; β_0 is the intercept; LT and PS represent the number (time-lagged by t° years) of stocked lean lake trout and Pacific salmon, re-

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spectively; β_{1i} and β_{2j} are the regression coefficients of the stocked salmonid terms (LT and PS) included in the model and indexed by i and j (to m and n number of regressor terms); and ε is an error term. Lake trout can live as long as 20 years or more (Martin and Olver 1980), while the Pacific salmon stocked in Lake Superior do not persist beyond about 5 years (Scott and Crossman 1973; Groot and Margolis 1991; Busby et al. 1996). In this modeling approach, the host species are time-lagged to account for growth needed to become eligible as preferred hosts. Thus, we considered lean lake trout lagged from 3 to 15 years and Pacific salmon lagged from 2 to 5 years as potential predictors.

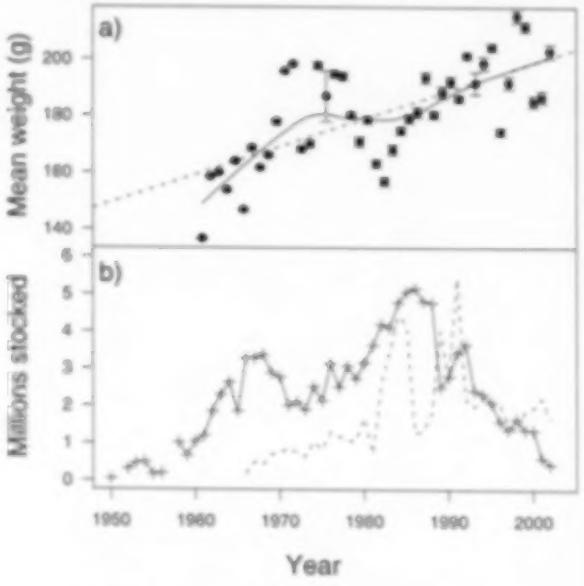
Given the uncertainty in the lagged terms to be included in the model and the potential for bias in traditional model selection methods (e.g., Olden and Jackson 2000), we employed BMA to aid in model selection. This technique has been used extensively in recent years in many disciplines and applications (Volinsky et al. 1997; Hoeting et al. 1999; Fernández et al. 2001), and its merits in an ecological context have been described in detail elsewhere (e.g., Wintle et al. 2003). A concise summary follows. Using a traditional frequentist stepwise procedure to identify the "best" model, there are a potential of 2^k models to be evaluated, where k is the number of potential predictor variables. Thus, the potential model space (2^k) can be quite large if $k = 17$ potential regressors, as in this analysis. BMA assumes that because of model uncertainty, there is not one best model; instead, multiple models may describe the relationship in the data with varying accuracy. BMA evaluates models in the 2^k model space and determines a posterior model probability, i.e., the probability of a model given the data or $\Pr(M_i|\text{data})$, for each model selected based on certain criteria, where M_i is the ith model from the model space. We used a uniform prior, meaning that each model was equally likely.

The criteria used for selecting models is based on two principles. First, when two nested models are compared and the simpler model is rejected based on its fit to the data, then all submodels of the simpler model are rejected. The second principle is based on occam's window, which concerns a ratio of the posterior model probabilities (Madigan and Raftery 1994; Hoeting et al. 1999). It removes models based on the outcome of a posterior probability ratio, $\Pr(M_0|\text{data})\cdot\Pr(M_1|\text{data})^{-1}$, where M_0 is the smaller model derived from M_1. If there is evidence for the smaller model, then the larger model is rejected. If the evidence is inconclusive (i.e., ratio ~1), then both models are kept. We set the criteria for accepting the larger model if the ratio was at least 1:20. These criteria identify a more parsimonious set of models and may improve predictive performance (Hoeting et al. 1999). We adapted the BMA function `bicreg` (Hoeting et al. 1999) for use in R (R Development Core Team 2004).

We used the models selected by BMA to find annual posterior-weighted estimates of mean sea lamprey weight and to find the predicted year 2004 mean weight. We extracted annual fits and the 2004 mean weight predictions from each model and used the corresponding posterior model probabilities to weight these fits according to the following:

$$(2)\qquad P_p(y|x, n_{\text{models}}) = \sum_{i=1}^{n} \phi_i P_{pi}(y_i|x_i, \text{data})$$

Fig. 1. (*a*) Observed mean sea lamprey (*Petromyzon marinus*) weights derived from multiple capture methods with a linear trend line (broken line: $F = 36.76$, $p < 0.001$, $R^2 = 0.473$) and a loess curve (solid line). Error bars represent ±1 SE. (*b*) Stocking rates of lean lake trout (*Salvelinus namaycush*) (solid line, crosses) and Pacific salmon (*Oncorhynchus* spp.) (broken line).



where $P_p(y|x, n_{\text{models}})$ is the posterior-weighted estimation of weight, $P_{pi}(y_i|x_i, \text{data})$ is the ith model's prediction given the regressors (the x_is) in that model and the data, and ϕ_i is the ith model's posterior probability.

Results

Trends in sea lamprey weight fluctuated but generally increased during the period 1961–2003. A linear fit to the data showed a highly significant increase in weight through the period 1961–2003 (Fig. 1*a*). Weights increased by nearly 1 g·year^{-1} during the period (weight = 0.9681 × year; $F = 36.76$, $p < 0.001$, $R^2 = 0.473$). The loess curve of the mean weights increased from 1961 to the mid-1970s, leveled off, decreased slightly during the early 1980s, and then increased from the late 1980s through 2003 (Fig. 1*a*). The two increases in weight roughly corresponded to time-lagged increases in lean lake trout and Pacific salmon stocking rates (Fig. 1*b*).

BMA identified 91 models that explained the relationship between sea lamprey weight and salmonid stocking. The small differences between the models' posterior probabilities indicated a high degree of uncertainty (Table 1) (Wintle et al. 2003). The top 25 models, listed by the rank of their posterior probabilities, are shown in Table 1. BMA analysis calculated the mean values of the coefficients across the 91 models and their probabilities of not being equal to zero given the data (Table 2). The intercept was included in all models, and BMA indicated that LT_{t-3}, LT_{t-9}, LT_{t-11}, and LT_{t-13} were important to sea lamprey weight based on their high probabilities of their coefficients not being equal to

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Table 1. Top 25 of 91 models ranked by their posterior model probabilities from Bayesian model averaging of sea lamprey (*Petromyzon marinus*) weight with salmonid stocking as predictors (LT, lean lake trout (*Salvelinus namaycush*); PS, Pacific salmon (*Oncorhynchus* spp.)) lagged from year t by the number of years indicated.

Model rank	Intercept	LT_{t-3}	LT_{t-4}	LT_{t-7}	LT_{t-8}	LT_{t-9}	LT_{t-11}	LT_{t-13}	LT_{t-14}	PS_{t-2}	PS_{t-3}	PS_{t-4}	PS_{t-5}	$Pr(M_j \mid data)$
1	✓	✓				✓	✓	✓	✓			✓		0.04577
2	✓	✓				✓	✓	✓	✓	✓		✓	✓	0.03947
3	✓			✓		✓		✓						0.03528
4	✓	✓				✓	✓	✓	✓					0.03495
5	✓	✓				✓	✓	✓				✓		0.03405
6	✓			✓		✓	✓	✓						0.02550
7	✓					✓	✓	✓						0.02550
8	✓	✓				✓								0.02414
9	✓	✓				✓						✓		0.02371
10	✓			✓		✓	✓	✓		✓				0.02051
11	✓	✓				✓	✓	✓						0.01761
12	✓	✓				✓						✓		0.01539
13	✓			✓		✓	✓	✓		✓				0.01539
14	✓	✓				✓	✓	✓	✓	✓		✓		0.01435
15	✓					✓	✓	✓						0.01434
16	✓	✓			✓	✓	✓	✓				✓		0.01394
17	✓	✓		✓		✓	✓	✓						0.01356
18	✓	✓				✓	✓	✓	✓	✓			✓	0.01305
19	✓	✓		✓		✓								0.01279
20	✓	✓	✓			✓	✓	✓	✓			✓		0.01244
21	✓	✓				✓	✓	✓		✓		✓		0.01230
22	✓	✓				✓							✓	0.01208
23	✓	✓		✓		✓		✓						0.01185
24	✓	✓				✓	✓	✓	✓					0.01170
25	✓	✓				✓	✓	✓	✓		✓	✓		0.01133

Table 2. Intercept and regressor coefficient mean values determined by Bayesian model averaging of sea lamprey (*Petromyzon marinus*) weight with salmonid stocking as predictors ranked by their probability (averaged over 91 models) of not being equal to zero.

Parameter	Mean coefficient value	SE	$Pr(\beta \neq 0 \mid data)$
Intercept	171.079	17.311	1.0
LT_{t-0}	7.4668×10^{-6}	3.9184×10^{-6}	0.9
LT_{t-3}	-4.1386×10^{-6}	3.5572×10^{-6}	0.7
LT_{t-13}	5.6504×10^{-6}	4.5847×10^{-6}	0.7
LT_{t-11}	-3.3100×10^{-6}	3.9089×10^{-6}	0.5
PS_{t-4}	1.4562×10^{-6}	2.1681×10^{-6}	0.4
LT_{t-7}	-1.3810×10^{-6}	2.7470×10^{-6}	0.3
LT_{t-14}	-1.3663×10^{-6}	2.9594×10^{-6}	0.2
PS_{t-2}	5.1135×10^{-7}	1.4076×10^{-6}	0.2
PS_{t-5}	6.2958×10^{-7}	1.6499×10^{-6}	0.2
LT_{t-8}	-7.0851×10^{-8}	1.6827×10^{-6}	0.1
LT_{t-10}	-1.1614×10^{-7}	1.0494×10^{-6}	0.1
PS_{t-3}	7.8465×10^{-8}	5.5759×10^{-7}	0.1
LT_{t-4}	-1.1731×10^{-8}	7.2249×10^{-7}	0.0
LT_{t-5}	-8.9882×10^{-8}	7.6956×10^{-7}	0.0
LT_{t-6}	-4.6775×10^{-9}	1.8531×10^{-7}	0.0
LT_{t-12}	1.7641×10^{-8}	1.4235×10^{-6}	0.0
LT_{t-15}	8.7781×10^{-9}	2.7452×10^{-7}	0.0

Note: The salmonids comprised lean lake trout (*Salvelinus namaycush*) (LT) and Pacific salmon (*Oncorhynchus* spp.) (PS) lagged from year t by the number of years indicated.

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Fig. 2. Model fits (line) from Bayesian model averaging of sea lamprey (*Petromyzon marinus*) weight and salmonid (*Oncorhynchus* spp.) stocking and observed sea lamprey weight (solid circles). The year 2004 predicted weight (open circle) is bounded by ±1 SD of the raw (nonposterior weighted) predictions of 2004 sea lamprey mean weight.

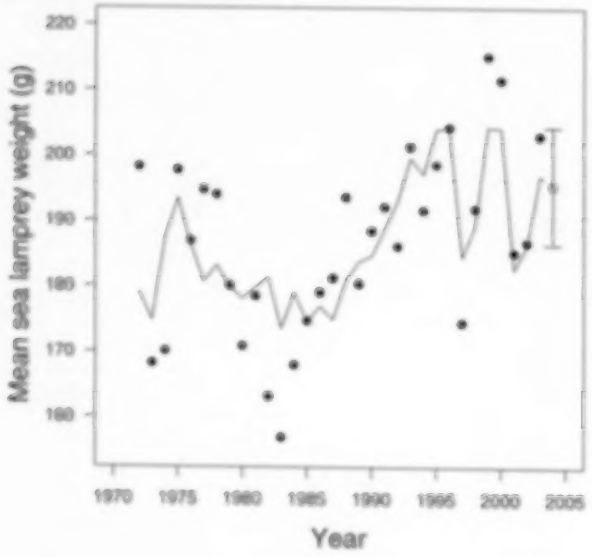
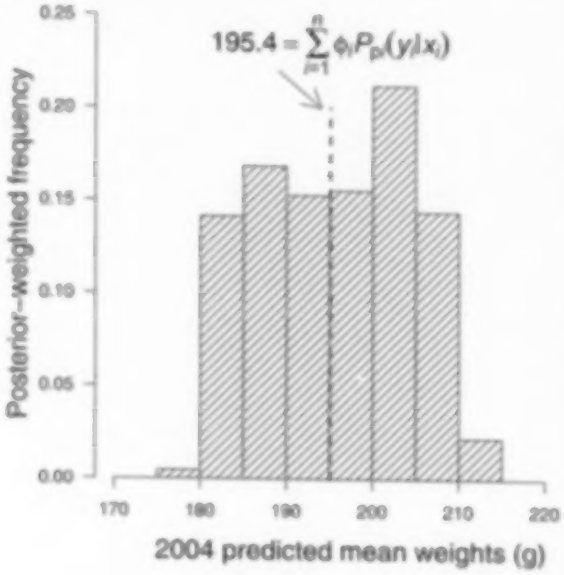


Fig. 3. Posterior-weighted frequency distribution of year 2004 predicted mean sea lamprey (*Petromyzon marinus*) weight. Predictions are weighted by their posterior model probabilities, and the mean posterior prediction, P_p, is indicated by the broken line.



zero (Table 2). Other regressors that may have had an effect on weight included PS_{t-4} and LT_{t-7}. These terms made up the components of some or all of the top five models with the highest posterior probability (Table 1). Also of note were LT_{t-13}, PS_{t-2}, and PS_{t-5} that were included in some of the 91 models identified through BMA. Other coefficients also somewhat important were LT_{t-9}, LT_{t-10}, and PS_{t-3}. There were six other potential regressors that were considered in the model space; however, the probabilities that these coefficient values were equal to zero were high (Table 2).

Using the models identified in BMA, we compared the averaged models' fit with the observed mean sea lamprey weight (Fig. 2) and used the posterior-weighted model predictions to estimate sea lamprey weight for 1 year ahead, 2004. The models' fit followed the general trends of the observed data (Fig. 2). Weight prediction estimates for 2004 from the 91 models ranged from 177.8 to 212.6 g. The posterior-weighted prediction estimate was 195.4 ± 9.0 g (1 SD) (Figs. 2 and 3).

Discussion

The size of sea lampreys increased through the period from 1961 to 2003 and, in particular, size has increased during the period since the last study (Houston and Kelso 1991). This increase was observed despite the potential for size-selective differences in capture methods. Mean sizes obtained from mechanical and electrical barrier catches appeared to be larger than those from portable trap catches in years when the two collection methods overlapped (M. Henson, US Fish and Wildlife Service, Marquette Biological Station, 1924 Industrial Parkway, Marquette, MI 49855, USA, unpublished data). Mean sizes after 1979 were exclusively based on portable traps, which, if mean size was underestimated during this period, may imply that the size increase trend may be more pronounced than that presented here.

The exception to the size increase trend that occurred from the mid-1970s to the early 1980s and the reasons for it are unclear. Stocking rates of lean lake trout declined from the late 1960s to the early 1970s, which might account for the size drop. However, Pacific salmon stocking increased during this period, which might suggest that size should have increased. Lake trout catch per effort in Michigan waters of Lake Superior during the size decrease period suggests that lean lake trout were near pre-sea lamprey invasion and pre-overharvest abundance levels (Wilberg et al. 2003). There is some evidence that lamprey abundance may have been declining during this period (Heinrich et al. 1980). If size is a density-dependent characteristic in sea lampreys, then it should have increased in these years. Thus, this period represents an unexplained anomaly in an otherwise long-term trend of increasing sea lamprey size.

Upon visual inspection, sea lamprey size peaks appeared to have some relationship with peaks in stocking events, offset by some number of years. BMA revealed that, of the variables considered as potentially important to sea lamprey size, stocked lean lake trout lagged 3, 9, 11, and 13 years stand out as most important. The coefficient for lake trout lagged 9 years had the highest probability of not being equal to zero. Lake trout stocked in the current year are primarily from the previous brood year-class. Thus, age-10 lake trout were important hosts for sea lampreys. Lean lake trout terms lagged more than 9 years may reflect the importance of re-

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cruits from stocked fish. As stocked fish mature and reproduce, their offspring grow and become potential host resources for sea lampreys. Significant long-lagged stocking terms may reflect a long interaction memory between sea lampreys and host species (Franklin 1989; Magnuson 1990). Age-4 lean lake trout (LT_{t-3}), which were also a significantly important regressor, are not likely the preferred hosts of large sea lampreys, but they may be very important to newly transformed sea lampreys and more abundant than older (larger) lean lake trout.

Age-5 Pacific salmon (PS_{t-4}) were also a significant regressor in many of the models and appeared in three of the five models with the highest posterior probabilities. BMA indicated that Pacific salmon lagged 4 (and to a lesser extent 5) years may be important to sea lamprey weight. Stocked Pacific salmon predictors were composites of three species, two of which mature in about 2 years (Chinook and coho) and another maturing in approximately 5 years (steelhead). Since the significant lag for Pacific salmon was 4 years, this indicated that Chinook and coho salmon, with a shorter life history, may not be as important to sea lamprey weight as the longer lived steelhead. However, Chinook and coho recruits from naturally reproducing stocked fish might have contributed to the 4-year time lag significance.

Sea lamprey size information included 50 years of data, and stocking information has been tabulated for 54 years, but time lags and missing data removed years available for model fitting. Furthermore, systematic stocking of Pacific salmon did not begin until 1967. This restricted model fitting to the years where the predictors had overlapped data (32 years) and thus permitted only a one-step ahead (year 2004) forecast prediction of size.

There were other potential predictors that were considered but could not have been used in model fitting. As with multiple linear regression analysis, regressors are considered for model fitting based on their biological meaningfulness. For example, there are regressors that express host abundance and community composition besides stocking rates, such as commercial catch per effort of salmonids. Although efforts to standardize catch per effort have been made for Michigan waters of Lake Superior (Wilberg et al. 2003), the models considered here would have required lake-wide catch per effort estimates given the assumption of opportunistic selection of streams by sea lampreys for spawning (Bergstedt and Seelye 1995). Future modeling would benefit from incorporating lake-wide catch per effort estimates, since these figures would more accurately reflect host availability than stocking rates. The focus on stocking rates ignores differential survival of stocked cohorts and recruitment from naturalized and wild fishes. Another potential regressor was sea lamprey abundance estimates, which have been calculated for Lake Superior for some years. Currently, however, lake-wide estimates begin for the year 1980 (J. Adams, US Geological Survey, Great Lakes Science Center, 1451 Green Road, Ann Arbor, MI 48105, USA, unpublished data), and past years' estimates are readjusted each time an estimate is made for the current year (D. Cuddy, Department of Fisheries and Oceans, Sea Lamprey Control Centre, 1 Canal Drive, Sault St. Marie, Ont., personal communication). As lake-wide reliable salmonid catch per effort and sea lamprey abundance estimates become available, we suggest that they be included in future BMA model fitting. Focusing on salmonid stocking rates also ignores other potential host species hypothesized to be less important to sea lampreys, such as siscowets (*Salvelinus namaycush siscowet*), a deepwater morphotype of lake trout, burbot (*Lota lota*), lake whitefish (*Coregonus clupeaformis*), lake herring (*Coregonus artedi*), or suckers (family Castostomidae), of which there is very little or no long-term lake-wide data available.

Although the efforts to suppress sea lamprey abundance in Lake Superior and to restore the fish community have been largely successful, the size trend is alarming. Average adult spawning landlocked sea lampreys in Lake Superior are as little as one fifth the size of anadromous sea lampreys (Davis 1967; Yergeau 1983; Halliday 1991). If their anadromous conspecifics are indicative of the size potential of landlocked Great Lakes sea lampreys, they are capable of growing to sizes much larger than those attained since their invasion. Sea lampreys in Lake Superior are typically 50–100 g smaller than sea lampreys in the other Great Lakes (e.g., GLFC annual reports; http://glfc.org/pubs_out/annualreports.php). As sea lampreys grow to larger sizes, their increased fecundity could offset some of the benefits of sea lamprey control (Manion 1972; Manion and Hanson 1980; Houston and Kelso 1991). Larger sea lampreys consume more blood and have a higher feeding rate (Kitchell and Breck 1980; Kitchell 1990) and cause increases in the host size death threshold (Kitchell 1990; Jorgensen and Kitchell 2005). In general terms, this sets the stage for decreases in the survival of host species (Farmer et al. 1977; Kitchell and Breck 1980; Swink 2003) and increases in the host death threshold size (Jorgensen and Kitchell 2005). The size increase trend, coupled with lower growth rates of lake trout as their populations have recovered (M. Ebener, 179 W. Three Mile Road, Sault Ste. Marie, MI 49783, USA, personal communication,), adds to the concern over lake trout mortality caused by to sea lamprey attacks.

What conclusions for management action can be drawn from sea lamprey size trends and the potential relationship they have with salmonid stocking? Among the goals set by Lake Superior stakeholders is to "rehabilitate and maintain a diverse, healthy, and self-regulating fish community, dominated by indigenous species and supporting sustainable fisheries" and for naturally reproducing Pacific salmon to be supplemented "by stocking that is compatible with restoration and management goals for indigenous fish species" (Horns et al. 2003). Pacific salmon sport fisheries are an important economic activity for many communities that surround the Great Lakes, and stocking will likely continue in the future (Goddard 2002).

Managers are, however, reevaluating stocking levels. They have reduced salmonid stocking because the lean lake trout has probably reached historical abundance levels and salmonid growth has slowed, likely as a consequence of overstocking (Bronte et al. 2003; Wilberg et al. 2003). According to the modeling results presented here, stocking Pacific salmon may have had a significant effect on sea lamprey size. Sea lampreys are able to grow to larger sizes more easily feeding on Pacific salmon than on lean lake trout (Jorgensen and Kitchell 2005). The larger observed sizes of sea lampreys in the other Great Lakes exemplify the effect of increasing the relative abundance of Pacific salmon

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in the Lake Superior fish community: more opportunities for sea lampreys to reach larger sizes. Pacific salmon have established naturalized reproducing populations that without further supplementation from hatchery-reared fish would still likely support a sport fishery, although perhaps at a reduced level.

To mitigate the potentially damaging effects of salmonid stocking while trying to achieve their stated goals, managers should also focus on strategies that provide alternative hosts to deflect sea lamprey induced mortality on lean lake trout and Pacific salmon. These alternative hosts should preferably be cold-water fishes that are undesirable commercially and recreationally and that can sustain sea lamprey predation pressure without significantly altering their abundance or ecological roles in the fish community. Such a list of alternative host fishes would include siscowets and burbot. Currently, siscowets and burbot are at or near historically high densities (Bronte et al. 2003). These fishes could serve as buffers; siscowets very likely do now, given their high abundance and their observed sea lamprey wounding rates (Bronte et al. 2003). Thus, maintaining these species at their current abundance would provide alternative host opportunities for sea lampreys that inhabit the colder waters, and they could inhibit sea lamprey growth.

Any management action may have unintended or unpredictable consequences. The effects of decreased Pacific salmon stocking on Lake Superior sea lamprey size are not known. There is evidence that suggests that sea lampreys cannot easily reach large sizes feeding only on lean lake trout (Jorgensen and Kitchell 2005). However, the effect on sea lamprey size from a shift in the salmonid community is unclear. A decrease in Pacific salmon stocking may have unintended consequences on the forage fish populations (Conner et al. 1993; Negus 1995; Kitchell et al. 2000). For example, Lake Superior salmonid diets are dominated by the nonnative rainbow smelt (*Osmerus mordax*), and because Pacific salmon growth trajectories are faster than those of lean lake trout, a reduction in Pacific salmon may lead to a reduction in predation pressure on rainbow smelt. This could release rainbow smelt and lead to interspecific competition for zooplankton between rainbow smelt and juvenile lake herring, which could cause poor lake herring recruitment (Anderson and Smith 1971). Therefore, any management actions with respect to Pacific salmon reductions therefore must be carefully considered. Attention should first focus on current levels of Pacific salmon natural recruitment, which should then influence future stocking decisions.

The fish community objectives state that Pacific salmon should be supplemented to keep a healthy fishery and that sea lampreys should cause insignificant mortality (<5%) of their prey species (Horns et al. 2003). Nevertheless, a relationship exists between sea lamprey size and Pacific salmon. Thus, there may be a trade-off for managers trying to reach the goals for the Lake Superior fish community. By stocking Pacific salmon, managers may be unintentionally subsidizing sea lamprey production, achieving one goal at the expense of another.

Acknowledgements

We thank M. Fodale, M. Henson, and R. Kahl (US Fish and Wildlife Service, Marquette Biological Station, Marquette, Michigan) for providing sea lamprey size data. S. Carpenter, I. Kaplan, and B. Yandell provided statistical assistance. We are grateful to S. Carpenter, I. Kaplan, L. Schubert, and an anonymous reviewer for valuable comments on an earlier version of this article. This research was supported in part by the US Department of Commerce, National Oceanic and Atmospheric Administration, Sea Grant College Program award No. NA16RG2257, the State of Wisconsin, and an Anna Grant Birge Memorial Award.

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Early life history of Lake Michigan alewives (*Alosa pseudoharengus*) inferred from intra-otolith stable isotope ratios

Elise Dufour, William P. Patterson, Tomas O. Höök, and Edward S. Rutherford

Abstract: We apply a robotic micromilling technique to the sampling of young-of-the-year (YOY) and 1+ otoliths from alewives (*Alosa pseudoharengus*) captured in different habitat types of Lake Michigan during 2001–2003. $\delta^{18}O$ values of otolith cores of YOY alewives from Muskegon Lake (a sheltered, drowned river mouth lake connected to Lake Michigan), Muskegon Channel (which connects Muskegon Lake to Lake Michigan), and Lake Michigan proper are compared with $\delta^{18}O$ values of ambient water from different potential early life habitats. Otolith core $\delta^{18}O$ values used in conjunction with $\delta^{13}C$ values serve as good discriminators of nursery areas. The majority of YOY alewives captured in Muskegon Lake emerge and grow in this habitat, whereas the majority of YOY alewives captured in Lake Michigan emerge and grow in Lake Michigan. In addition, early-life movements of alewives between the two lakes are documented but limited. Even if drowned river mouth lakes are more favorable for alewife growth and survival, their contribution to the Lake Michigan population could be limited because all of the 1+ alewives were individuals that spent their early life in Lake Michigan. The application of high-resolution isotope analysis of small otoliths of forage fish for fish population dynamics studies appears promising.

Résumé : Nous avons appliqué un système automatisé de microfraisage à l'échantillonnage d'otolithes de gaspareaux (*Alosa pseudoharengus*) jeunes de l'année et 1+ pêchés dans différents types d'habitats du Lac Michigan durant la période 2001–2003. Nous avons comparé les valeurs de $\delta^{18}O$ des centres des otolithes de jeunes de l'année pêchés dans le lac Muskegon, dans le Muskegon Channel (qui relie le lac Muskegon au lac Michigan) et dans le lac Michigan aux valeurs de $\delta^{18}O$ de l'eau environnementale prélevée dans différentes zones potentielles de grossissement des alevins. Les valeurs de $\delta^{18}O$ des centres des otolithes utilisées conjointement aux valeurs de $\delta^{13}C$ des otolithes permettent de discriminer les lieux de grossissement potentiels. La majorité des jeunes de l'année capturés dans le lac Muskegon est originaire de ce lac et y a grandi, alors que la majorité des jeunes de l'année capturés dans le lac Michigan est originaire de ce dernier. Même si des changements d'habitats des alevins entre le lac Muskegon et le lac Michigan sont détectés, ils demeurent limités. Enfin, les gaspareaux 1+ capturés dans le lac Michigan étaient des individus ayant passé leur première saison de croissance dans ce lac. Bien que les conditions d'habitat apparaissent plus favorables à la croissance et à la survie des gaspareaux dans le lac Muskegon, leur contribution à la population du lac Michigan pourrait donc n'être que limitée. Le microfraisage s'avère adapté à l'étude des otolithes de petite taille, et cette technique apparaît très prometteuse pour documenter la dynamique des populations des espèces fourrage.

Introduction

The alewife (*Alosa pseudoharengus*) population is currently a critical component of Laurentian Great Lakes ecosystems, providing the forage base for a diverse salmonine predator community that supports valuable sport and commercial fishing. Simultaneously, alewives are capable of restructuring zooplankton communities and suppressing native planktivores. Since its invasion during the 1940s the alewife population in Lake Michigan has fluctuated dramatically

Received 14 December 2004. Accepted 19 April 2005. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on 27 September 2005.
J18455

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 doi: 10.1139/F05-147 © 2005 NRC Canada

owing to variable recruitment and predation by salmonines (Jude and Tesar 1985; Madenjian et al. 2002, 2005). A better understanding of alewife recruitment processes should aid in the management of the entire ecosystem. During the spring and summer, alewives migrate inshore to spawn in a variety of habitat types (e.g., drowned river mouth lakes, harbors, embayments, tributaries, and nearshore coastal areas of Lake Michigan proper). Because certain sheltered habitat types (e.g., drowned river mouth lakes, harbors, and embayments) are productive and relatively warm, they have been hypothesized to be more favorable for early-life alewife growth compared with exposed shorelines. Therefore, young-of-the-year (YOY) individuals hatched in such habitats may contribute significantly to alewife year classes.

Dramatic improvements have been made in the range of techniques used in the study of spatial behavior of fishes in natural environments, including population genetics, parasite loads, mark–recapture and hydroacoustic studies, and more recently microchemistry of otolith carbonate (Campana 1999; Lucas and Baras 2000). Teleost otoliths are accretionary calcium carbonate structures usually in the form of aragonite laid down on an organic matrix (Carlström 1963; Degens et al. 1969). The use of otolith chemistry as a record of fish life history is based on three key properties. First, otoliths begin to form near the time of hatching and grow throughout the entire life of a fish, displaying a regular series of growth increments (Campana and Nielson 1985). Second, they remain metabolically inert (i.e., aragonite is not reworked once deposited). Third, elements deposited onto the growing otolith reflect the physical, chemical, and biological environments experienced by individual fish (e.g., Campana 1999). $\delta^{18}O$ values of otolith aragonite ($\delta^{18}O_{oto}$) reflect $\delta^{18}O$ values of the ambient water ($\delta^{18}O_w$), as modified by ambient temperature (Kalish 1991; Patterson et al. 1993; Thorrold et al. 1997). In contrast with oxygen, carbon isotopes in otolith aragonite ($\delta^{13}C_{oto}$) are deposited in isotopic disequilibrium with the ambient water (e.g., Iacumin et al. 1992) because 20%–30% of the carbon in otolith is derived from metabolic processes (Tohse and Mugiya 2002; Høie et al. 2003; Wurster and Patterson 2003). However, otoliths can still record geographic differences in $\delta^{13}C$ of dissolved inorganic carbon ($\delta^{13}C_{DIC}$) (Thorrold et al. 1997; Weidman and Millner 2000). Therefore, geographic variation in isotope ratios of the environment can be used as an indicator of home range, natal origin, nursery habitat, and spatial distribution of a fish stock or species (e.g., Northcote et al. 1992; Campana 1999).

We hypothesized that the differences in physical, chemical, and biological conditions in different Lake Michigan habitat types would lead to significant variations in $\delta^{18}O_w$ and $\delta^{13}C_{DIC}$, which should translate into $\delta^{18}O_{oto}$ and $\delta^{13}C_{oto}$ variations in alewife otoliths. Because we want to reconstruct alewife early life, a technique that enables the recovery of $\delta^{18}O_{oto}$ and $\delta^{13}C_{oto}$ values at the otolith core is needed. New developments in robotic microsampling and mass spectrometry techniques (Wurster et al. 1999) have been combined with the chronological record of growth increments to provide unprecedented high-resolution environmental and biological records from stable isotope values. Analysis of pen-reared Atlantic cod (*Gadus morhua*) otoliths by Høie et al. (2004*a*) demonstrated that only high-resolution sampling (at an intra-seasonal scale) could accurately extract the true temperature range experienced by fish. This methodology has been successfully applied toward the reconstruction of thermal histories of modern and archaeological fishes (Patterson 1998; Wurster et al. 2005). However, stable isotope profiles of otoliths at an intra-seasonal scale have not been specifically applied toward studies of spatial behavior or to YOY otoliths.

This study represents the first attempt to apply high-resolution sampling of small otoliths from forage fish to the study of early life history. We analyzed cores of YOY alewives captured during the fall of 2002 in Muskegon Lake (a sheltered, drowned river mouth lake connected to Lake Michigan) and compared them with cores of YOY and yearling alewives from other Lake Michigan habitat types captured during 2001–2003. The objectives of this study were to (*i*) evaluate whether $\delta^{18}O_{oto}$ and $\delta^{13}C_{oto}$ of cores can discriminate nursery areas of YOY alewives in Lake Michigan, (*ii*) document the occurrence and frequency of movement between habitats by alewives during their first season of growth, and (*iii*) infer the relative contributions of alewife recruits from the different habitat types.

Material and methods

Water and fish collection

Seventy-three water samples (30–500 mL) were collected and stored in Nalgene™ bottles for $\delta^{18}O_w$ analysis (Table 1). During 2002, geographic and depth variation in $\delta^{18}O_w$ was evaluated by collecting water in Lake Michigan (off Sturgeon Bay and near Muskegon Lake) and in three drowned river mouth lakes adjacent to Lake Michigan (Muskegon Lake, Pigeon Lake, and Manistee Lake) (Fig. 1). To assess both spatial and temporal variability, water samples were also collected in various habitats during 2003 (although no YOY born this year were isotopically analyzed).

From mid-May to November of 2001 and 2002, we compared physical and biological conditions in various drowned river mouth lakes and a nearshore coastal zone of Lake Michigan and simultaneously collected age-0 alewives from these habitats (Fig. 1). We focused our sampling on Muskegon Lake, an inland, drowned river mouth lake located along the east shoreline of Lake Michigan, the adjacent nearshore area of Lake Michigan, and Muskegon Channel (which connects Muskegon Lake to Lake Michigan). YOY alewives were captured in these habitats with bottom and midwater trawls. We also obtained YOY alewives collected by the US Geological Survey Great Lakes Science Center at one location in Lake Michigan (near Sturgeon Bay) using bottom trawls. To infer the nursery areas of these YOY alewives, we analyzed otoliths from specimens collected during September–November 2002. We completed our sampling of the 2002 year class by collecting overwintering yearling alewives in the adjacent nearshore area of Lake Michigan. Fish were captured using bottom trawls during June 2003.

For comparison, we also analyzed alewives belonging to the 2001 year class. We selected YOY that we collected in October in the nearshore area of Lake Michigan adjacent to Lake Muskegon as well as YOY that were collected in October in two locations of Lake Michigan (near Saugatuck and

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Table 1. $\delta^{18}O_w$ values (Vienna Standard Mean Ocean Water) collected in 2002 and 2003 in Muskegon Lake and other drowned river mouth lakes, as well as tributaries and different sites of Lake Michigan.

Site	Date of collection	$\delta^{18}O_w$ (‰) (depth (m)) Value(s)	Mean
Muskegon Lake			–8.6
Downstream area	9 July 2002	–8.9 (1), –8.8 (6)	
	15 July 2002	–8.7 (1), –8.7 (5)	
	27 Aug. 2002	–8.3 (1)	
	10 Sept. 2002	–8.2 (1), –8.2 (10)	
	24 Sept. 2002	–8.6 (1), –8.2 (8)	
	22 Oct. 2002	–8.2 (1)	
	18 Nov. 2002	–8.5 (2)	
	10 Oct. 2003	–8.7 (1)	
Upstream area	23 July 2002	–8.6 (2), –8.7 (5)	
	7 Aug. 2002	–8.6 (1), –8.6 (4)	
Lake Michigan, nearshore area adjacent to Muskegon Lake			–6.0
25–50 m depth contour	24 June 2002	–6.2 (1), –6.2 (15)	
	15 July 2002	–6.1 (1), –5.8 (10)	
	24 July 2002	–5.8 (1), –6.1 (10)	
	7 Aug. 2002	–5.9 (1), –5.9 (10)	
5–25 m depth contour	8 July 2002	–6.0 (1), –6.2 (10)	
	27 Aug. 2002	–5.9 (1), –5.9 (10)	
	9 Sept. 2002	–5.6 (1), –5.8 (17)	
	24 Sept. 2002	–6.1 (1), –6.2 (25)	
	22 Oct. 2002	–6.1 (1), –6.0 (37)	
	18 Nov. 2002	–5.8 (1)	
	10 Oct. 2003	–6.2 (1)	
Lake Michigan			
Sturgeon Bay	Oct. 2002	–6.3 (1)	
Hagar Park	10 Oct. 2003	–6.4 (1)	
Saugatuck (Oval Beach)	10 Oct. 2003	–6.3 (1)	
Ludington	10 Oct. 2003	–6.3 (1)	
Fisherman's Island State Park	2 Nov. 2003	–6.3 (1)	
Buffinton Harbor	11 Nov. 2003	–6.0 (1)	
Chicago	11 Nov. 2003	–5.9 (1)	
Zion	11 Nov. 2003	–6.1 (1)	
Milwaukee (McKinley Park)	11 Nov. 2003	–6.4 (1)	
Sheboygan	11 Nov. 2003	–6.2 (1)	
Green Bay	11 Nov. 2003	–6.5 (1)	
Menominee	11 Nov. 2003	–7.6 (1)	
Bay de Noc (Gladstone)	11 Nov. 2003	–7.3 (1)	
Manistique	11 Nov. 2003	–7.5 (1)	
Nanbinway	11 Nov. 2003	–7.0 (1)	
Point La Barbe	11 Nov. 2003	–6.8 (1)	
Manistee Lake			–10.9
South	9 July 2002	–11.1 (1)	
	23 July 2002	–10.9 (1)	
	13 Aug. 2002	–10.8 (1)	
Pigeon Lake			–7.3
Upper	30 July 2002	–7.3 (1)	
Lower	30 July 2002	–6.2 (1)	
Port Sheldon	10 Oct. 2003	–8.5 (1)	
Others			
Black River (South Haven)	10 Oct. 2003	–7.2 (1)	
Charlevoix Lake (south of Charlevoix)	2 Nov. 2003	–9.1 (1)	
Elk Lake (near Kewadin)	2 Nov. 2003	–9.5 (1)	
Escanaba River (Gladstone)	11 Nov. 2003	–11.0 (1)	
Fox River (Green Bay)	11 Nov. 2003	–6.8 (1)	
Kalamazoo River (Saugatuck)	10 Oct. 2003	–9.0 (1)	

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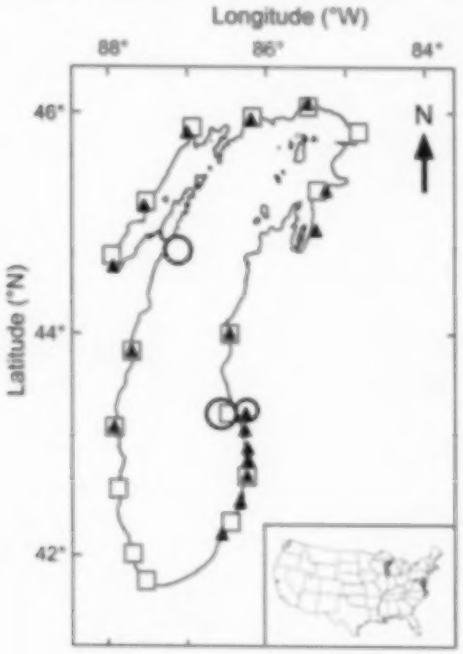
Table 1 (*concluded*).

Site	Date of collection	$\delta^{18}O_w$ (‰) (depth (m))	
		Value(s)	Mean
Lake Macatawa (Holland)	10 Oct. 2003	−6.2 (1)	
Manistique River (Manistique)	11 Nov. 2003	−11.5 (1)	
Menominee River (Marinette)	11 Nov. 2003	−10.6 (1)	
Millecoquins River (US-2)	11 Nov. 2003	−11.5 (1)	
Milwaukee River (Milwaukee)	11 Nov. 2003	−7.8 (1)	
Pere Marquette Lake (Ludington)	10 Oct. 2003	−10.5 (1)	
Sheboygan River (Sheboygan)	11 Nov. 2003	−8.1 (1)	
Spring Lake (Grand Haven)	10 Oct. 2003	−8.7 (1)	
St. Joseph River (St. Joseph)	10 Oct. 2003	−8.0 (1)	

Fig. 1. Map of Lake Michigan with the location of water and alewife (*Alosa pseudoharengus*) YOY and 1+ individuals collection. Squares, water collected from Lake Michigan; triangles, water collected from drowned river mouth lakes or tributaries; circles, alewife collection sites.

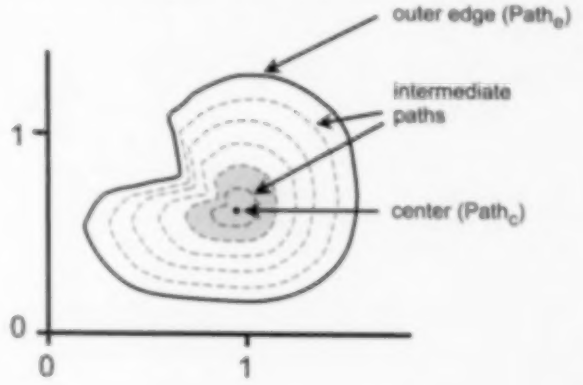


Sturgeon Bay) by the US Geological Survey Great Lakes Science Center (Fig. 1). We also selected yearlings that we collected in the same nearshore area of Lake Michigan and in Muskegon Channel in April and May 2002.

Otolith preparation and micromilling

YOY alewives were stored frozen in the field and transferred to the laboratory. At least three specimens from each location were selected for sagittal otolith removal and were dissected using fine probes. We used the computer-controlled apparatus described by Wurster et al. (1999) for micromilling YOY alewife otoliths. We adapted the sampling procedure to address several technical difficulties, including the small size and fragility of specimens. Originally,

Fig. 2. Microsampling of an alewife (*Alosa pseudoharengus*) otolith using a robotic micromilling apparatus. The center ($Path_c$) and the outer edge ($Path_e$) were digitized as two series of three-dimensional coordinates and interpolated using a cubic spline. Five intermediate sampling paths (IP) (broken lines) were then calculated. The core (shaded) represents the early life of the fish and was obtained by combining the powder collected during the milling of $Path_c$ with that of the two following IPs. The core represents the early life of a fish, whose isotope ratios were used to infer origin and potential habitat switch.



otoliths were directly glued onto glass slides for polishing and micromilling, but a number of them fractured during this process. Therefore, otoliths were subsequently placed in a plastic mold positioned on their inner side and embedded in an epoxy resin (Epofix Struers™). The resin was allowed to harden overnight at room temperature and then at 50 °C for an additional hour. Individual hardened blocks were removed and carefully polished by hand until growth bands and the nucleus of each otolith were revealed. Resin blocks were then cleaned in deionized water, dried, and glued onto glass slides.

Specimens were attached to a three-dimensional micropositioning stage under a fixed high-precision dental drill. For each specimen, the center ($Path_c$) and the outer edge ($Path_e$) were digitized in real time as two series of three-dimensional coordinates and interpolated using a cubic spline (Fig. 2). Subsequently, from four to nine intermediate sampling paths were calculated between $Path_c$ and $Path_e$. Core samples consisted of a mixture of the powder from

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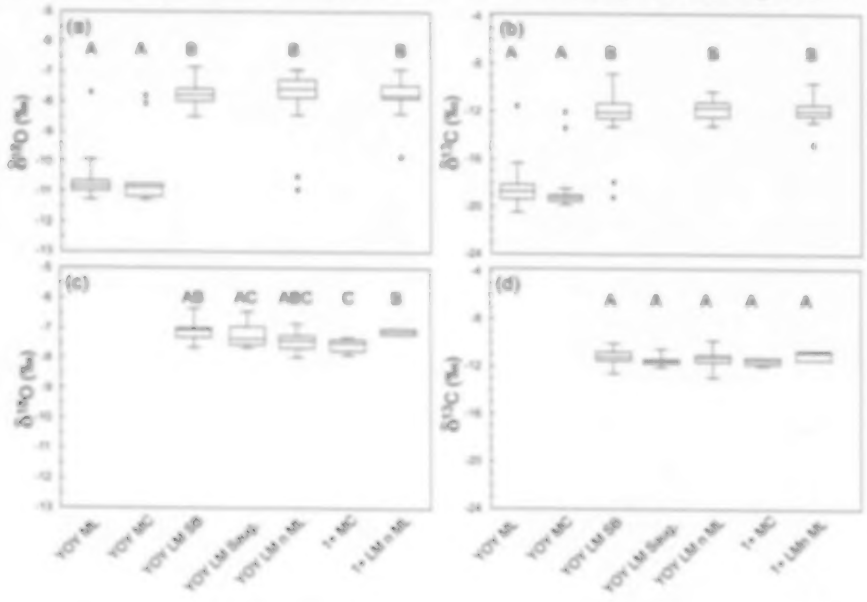
Fig. 3. Comparison of the core isotope ratios (‰; Vienna Pee Dee Belemnite) of alewife (*Alosa pseudoharengus*) groups (YOY and 1+ groups) captured in different habitats of Lake Michigan in (*a* and *b*) 2002 and (*c* and *d*) 2001. The upper and lower parts of the box represent 75% and 25% of the scores, respectively. The horizontal line inside the box represents the median. The end of the whiskers represents the largest and smallest values that are not outliers. Stars represent outliers. Different letters indicate significantly different values (Mann–Whitney, $p < 0.001$). ML, Muskegon Lake; MC, Muskegon Channel; LM SB, Lake Michigan offshore of Sturgeon Bay; LM Saug., Lake Michigan offshore of Saugatuck; LM n ML, Lake Michigan near Muskegon Lake.



Path$_x$ with that of the following two to four intermediate paths (Fig. 2). The theoretical mass of core samples, calculated by estimated powder volume and density, was ~10–25 µg.

Isotope analysis of water and otoliths

$\delta^{18}O_w$ values were determined using a continuous-flow pyrolysis technique. Aliquots of 1 µL of water were injected into a Finnigan MAT ThermoChemical Elemental Analyzer (TC/EA) via a GC PAL® autosampler. The resulting CO was analyzed with a ThermoFinnigan DeltaPlus XL via a ConFloIII interface. Values are reported in standard per mil notation relative to Vienna Standard Mean Ocean Water (VSMOW). Accuracy and precision were checked through the repetitive analysis of internal laboratory standards. Sample precision was determined to be ±0.3‰.

Otolith subsamples were stored in stainless steel cups and roasted in vacuo for 1 h at 200 °C. Stable oxygen and carbon isotope ratios were determined using a Finnigan MAT 252 (Department of Geology, Syracuse University, Syracuse, New York) or MAT 253 (Department of Geological Sciences, University of Saskatchewan, Saskatoon, Saskatchewan) directly coupled to a KIEL-III automated carbonate preparation device. The mass spectrometers were optimized to process CO_2 samples evolved from very small carbonate samples (>10–15 µg) of otolith cores. The mass of samples was ascertained by comparing the amount of CO_2 generated after reacting with phosphoric acid with that of precisely weighed NBS-19 standard samples. All measurements are reported in the standard delta notation (per mil, ‰) relative to the Vienna Pee Dee Belemnite standard. Accuracy and precision were checked by routine analysis of the NBS-19 standard and was determined to be ±0.1‰.

Results

The $\delta^{18}O_w$ values in which YOY alewives reside were determined for use as an indicator of retrospective habitat. $\delta^{18}O_w$ values range from –11.5‰ to –5.8‰ (Table 1). Multiple analyses of Muskegon Lake and the nearshore area of Lake Michigan adjacent to Muskegon Lake during the growing season of 2002 and between 2002 and 2003 exhibit no significant variation in $\delta^{18}O_w$ with depth or time of collection (Table 1). However, a significant spatial variability was observed. For example, drowned river mouths have lower $\delta^{18}O_w$ values than Lake Michigan, while large embayments like Green Bay have values similar to Lake Michigan (Table 1). This general pattern is not exhibited by habitats such as Lake Macatawa and Pigeon Lake, which present relatively high $\delta^{18}O_w$ values, whereas in Lake Michigan near Menominee and Manistique, $\delta^{18}O_w$ values are relatively low (Table 1).

A total of 207 valid $\delta^{18}O$ values and 215 valid $\delta^{13}C$ core values were obtained for 256 YOY and yearling alewife otoliths, demonstrating that high-resolution sampling can be applied to small otoliths of a forage fish species (Fig. 3). In 2002, $\delta^{18}O$ and $\delta^{13}C$ values for YOY ranged from –11.2‰ to –6.9‰ and from –20.9‰ to –9.0‰, respectively (Figs. 3*a* and 3*b*). A nonparametric Mann–Whitney test was used to

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compare fish groups because not all of them present a normal distribution. There were no significant differences in $\delta^{18}O_{otc}$ and $\delta^{13}C_{otc}$ values between YOY groups captured in Lake Muskegon and Muskegon Channel or among YOY groups captured from different locations in Lake Michigan (Mann–Whitney, $p < 0.001$) (Figs. 3*a* and 3*b*). On the contrary, otoliths from both Muskegon Lake and Muskegon Channel fish groups had significantly lower $\delta^{18}O_{otc}$ and $\delta^{13}C_{otc}$ compared with alewives captured in Lake Michigan. In 2001, YOY $\delta^{18}O_{otc}$ and $\delta^{13}C_{otc}$ values ranged from –8.0‰ to –6.4‰ and from –12.9‰ to –10.0‰, respectively (Figs. 3*c* and 3*d*). There was no significant difference among fish groups (Mann–Whitney, $p < 0.001$) (Figs. 3*c* and 3*d*).

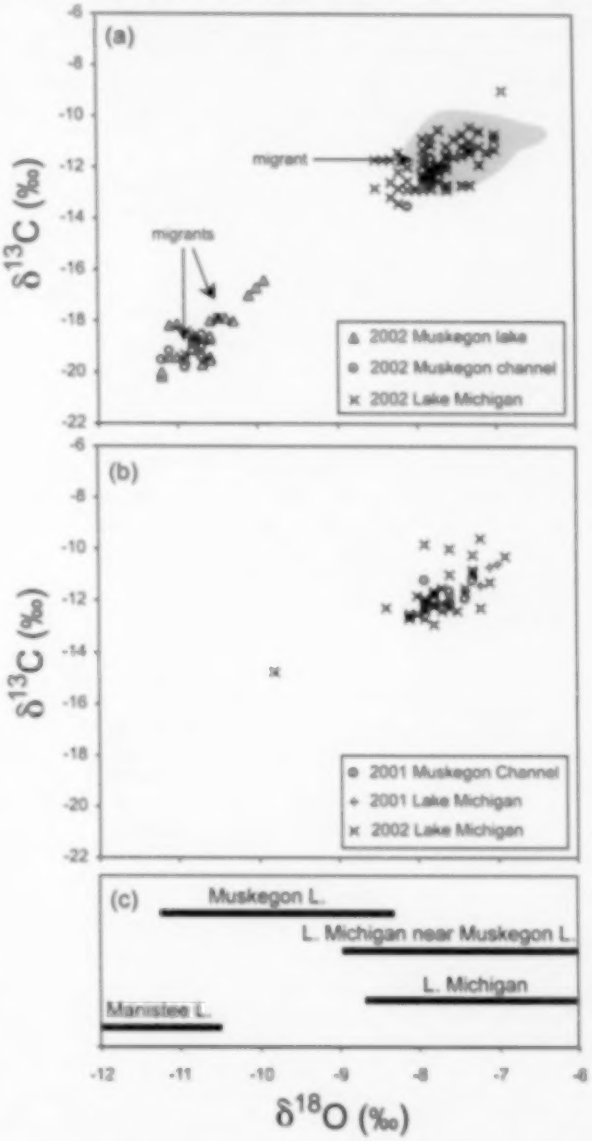
In addition to geographic differences, isotope variability was observed within YOY fish groups. Four groups contained outlier individuals in 2002 (Figs. 3*a* and 3*b*). One individual captured in Muskegon Lake and two individuals from Muskegon Channel exhibited values similar to those of Lake Michigan. Two individuals captured in the nearshore area of Lake Michigan exhibited values similar to those of Muskegon Lake. There were no outliers for fish born in 2001 (Figs. 3*c* and 3*d*). Statistical tests remained unchanged whether or not the outlier values were included.

$\delta^{18}O$ and $\delta^{13}C$ values for yearlings born in 2002 ranged from –9.8‰ to –6.9‰ and from –14.8‰ to –9.6‰, respectively (Figs. 3*a* and 3*b*). One 1+ individual captured in the nearshore area of Lake Michigan exhibited a lower $\delta^{18}O_{otc}$ value than the rest of the group (Fig. 3*a*). $\delta^{18}O_{otc}$ and $\delta^{13}C_{otc}$ values for yearlings born in 2001 ranged from –7.9‰ to –7.0‰ and from –11.9‰ to –10.6‰, respectively (Figs. 3*c* and 3*d*). The 1+ alewives from Muskegon Channel and the nearshore habitat of Lake Michigan fish are significantly different for $\delta^{18}O_{otc}$ values but not for $\delta^{13}C_{otc}$ values (Mann–Whitney, $p < 0.001$) (Figs. 3*c* and 3*d*).

Discussion

To evaluate whether variation of $\delta^{18}O_{otc}$ and $\delta^{13}C_{otc}$ values of otolith cores of alewives from different habitats of Lake Michigan can be attributed to different geographic origins, we characterized the isotope values of probable habitats. The few values available in the literature confirm the general pattern of variation in $\delta^{18}O_w$ among Lake Michigan habitat types. We observed a $\delta^{18}O_w$ value of –6.4‰ for a sample that we collected at Grand Traverse Bay in 1998, while during the early 1950s, Epstein and Mayeda (1953) measured a $\delta^{18}O_w$ value of –6.1‰ at an undisclosed Lake Michigan location. $\delta^{18}O_w$ values of tributaries of Lake Michigan analyzed by Coplen and Kendall (2000) displayed a range from approximately –12‰ to approximately –8‰. Although we documented spatial variation, we did not observe significant temporal variation in $\delta^{18}O_w$ within habitats over the growing season of YOY. This is to be expected given the size and the residence time of the habitats. Therefore, mean $\delta^{18}O_w$ values observed during 2002–2003 (–8.6‰ for Muskegon Lake, –6.0‰ for the nearshore area of Lake Michigan adjacent to Muskegon Lake, –6.3‰ for Lake Michigan, and –10.9‰ for Manistee Lake) are likely indicative of oxygen isotope signatures within these habitats during recent years (Table 1).

Fig. 4. (*a*) Plot of $\delta^{18}O$ and $\delta^{13}C$ values (‰; Vienna Pee Dee Belemnite) of YOY alewife (*Alosa pseudoharengus*) otolith cores collected during the fall of 2002 in different habitats of Lake Michigan. The range of $\delta^{18}O$ and $\delta^{13}C$ values of otolith cores of YOY alewives collected in 2001 is indicated by the shaded area. (*b*) Plot of $\delta^{18}O$ and $\delta^{13}C$ values (‰; Vienna Pee Dee Belemnite) of otolith cores of 1+ alewives from the 2001 and 2002 year classes collected in 2002 and 2003. (*c*) Estimation of potential ranges of $\delta^{18}O_{otc}$ values of otoliths crystallized in different origins calculated using measured $\delta^{18}O_w$ values and the equation of Patterson et al. (1993) and assuming potential temperature ranges experienced by fish in these habitats.



$\delta^{18}O_{otc}$ and $\delta^{13}C_{otc}$ values of otolith cores of YOY alewives captured in Muskegon Lake during 2002 were compared with values from alewives captured in other habitat types in 2001 and 2002. We observe a clear distinction be-

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tween $\delta^{18}O_{otc}$ and $\delta^{13}C_{otc}$ values of alewives collected from Muskegon Lake and those from Lake Michigan (Figs. 3 and 4). $\delta^{18}O_{otc}$ values are a function of ambient $\delta^{18}O_w$ values and temperature (e.g., Patterson et al. 1993; Thorrold et al. 1997). Therefore, we can predict the potential range of $\delta^{18}O_{otc}$ values of aragonite crystallized within each habitat using an aragonite temperature–fractionation relationship and mean $\delta^{18}O_w$ habitat values and assuming potential temperature ranges experienced by fish in these habitats (Fig. 4*c*). Multiple otolith-specific temperature–fractionation relationships have been developed from fish growing in well-constrained natural and laboratory environments (e.g., Patterson et al. 1993; Thorrold et al. 1997; Høie et al. 2004*b*). Although these relationships have slopes that are statistically indistinguishable from each other and from the inorganic aragonite temperature–fractionation relationship of Kim and O'Neil (1997), the intercepts differ significantly (Campana 1999; Thorrold and Hare 2002). We used the equation of Patterson et al. (1993) because it was specifically developed for freshwater fishes in natural systems, whereas the other equations were generated for marine fish. Additionally, the other equations have been noted to generate temperatures that may be 4 °C off the true temperatures of freshwater fishes raised in the laboratory and captured from well-constrained natural environments. A broad potential temperature range from +16 °C to +30 °C was estimated based on field temperature measurements (T.O. Höök, unpublished data) and laboratory experiments (Edsall 1970). Comparison between predicted and measured $\delta^{18}O_{otc}$ values suggests that YOY alewives captured in Muskegon Lake could have theoretically emerged in various drowned river mouth lakes including Muskegon Lake but not in Pigeon Lake or the majority of Lake Michigan sites (Fig. 4*a*). We were not able to measure the $\delta^{13}C_{DIC}$ values of the different habitats, but significant differences between drowned river mouth lakes and Lake Michigan are expected. The DIC originates from the atmosphere or the mineralization of organic matter, which have significantly different $\delta^{13}C$ values. The relative contribution of the two end members depends on lake size and is reflected by the positive relationship between $\delta^{13}C$ values of planktivorous fish tissues, including otoliths, and the size of the lake surface (Dufour 1999; Perga and Gerdeaux 2004). Drowned river mouth lakes such as Muskegon Lake have a smaller surface area compared with Lake Michigan (Muskegon Lake surface area is ~0.03% that of Lake Michigan) and thus should exhibit lower $\delta^{13}C_{DIC}$ values. Therefore, low $\delta^{18}O_{otc}$ and $\delta^{13}C_{otc}$ values of cores of YOY alewives captured in Muskegon Lake during the fall of 2002 are consistent with an early life spent in a small sheltered habitat, most likely Muskegon Lake.

Based on $\delta^{18}O_{otc}$ values, individuals captured in Lake Michigan during the fall of 2002 could have emerged in various habitats, including Muskegon Lake (Fig. 4*a*). Theoretically, it is not possible to unequivocally assign these fish to a precise origin because Lake Michigan itself contains, or is connected to, numerous habitats that could potentially exhibit large differences, and we did not measure $\delta^{18}O_w$ values within all of these habitats. However, the clear segregation between most alewives from Lake Michigan habitats and those of Muskegon Lake suggests different environmental conditions experienced during early life (Fig. 4*a*). Moreover, the combination of relatively high $\delta^{18}O_{otc}$ and $\delta^{13}C_{otc}$ values exhibited by most individuals captured in Lake Michigan is characteristic of open large lake habitats rather than drowned river mouth lakes or tributaries. Therefore, it is likely that the majority of YOY alewives captured in our lake-wide and nearshore study areas grew in Lake Michigan.

Although a large majority of surviving YOY captured during the fall of 2002 in Muskegon Lake grew in Muskegon Lake, we did find evidence of alewives moving between this drowned river mouth lake and Lake Michigan. One alewife captured in Muskegon Lake exhibited a value compatible with an early life spent in Lake Michigan (Fig. 4*a*). Individuals collected in Muskegon Channel had isotope values that suggest an early life in Muskegon Lake or Lake Michigan (Fig. 4*a*). In addition, movements of individual alewives into Lake Michigan are suggested by isotope values of two YOY alewives captured in Lake Michigan during the fall of 2002, which present values compatible with early growth in a small sheltered habitat (Fig. 4*a*). Even though it is not possible to unequivocally ascribe an origin to all fish because not all potential habitat origins have been characterized, these two alewives likely emerged and grew in a drowned river mouth (such as Muskegon Lake) or tributary and then moved to the Lake Michigan nearshore habitat. The presence of migrant specimens from drowned river mouth lakes within the Lake Michigan groups was not suggested for 2001 because no YOY individuals exhibited relatively low $\delta^{18}O_{otc}$ and $\delta^{13}C_{otc}$ values (Fig. 4*b*). Alewife habitat switches described by $\delta^{18}O_{otc}$ and $\delta^{13}C_{otc}$ core values are consistent with field observations that suggest that larvae and juveniles born in Lake Muskegon could be transported through advection or active swimming during early stages of development in Lake Michigan (T.O. Höök, unpublished data).

During the summers of 2001 and 2002, Muskegon Lake and other drowned river mouth lakes of Lake Michigan had warmer waters and greater densities of small-bodied zooplankton than Lake Michigan (T.O. Höök, unpublished data). Because these conditions are favorable for faster larval and juvenile growth rates than exposed shorelines of lakes, drowned river mouth lakes should yield a disproportionate (relative to their volume) number of recruits to the year class.

Similarly to YOY captured in the fall of 2002, a large majority of yearlings captured in the nearshore study area in the spring of 2003 were individuals that spent their early life in Lake Michigan (Fig. 4*b*). Only one individual exhibiting an intermediate $\delta^{18}O_{otc}$ value is likely to have spent at least part of early life in a different habitat, whereas no 1+ alewives captured in Muskegon Channel in 2002 exhibited values typical of an early life in Muskegon Lake (Fig. 4*b*). Therefore, all of the 2001 year-class fish and the majority of the 2002 year-class individuals likely emerged and grew in Lake Michigan (Figs. 4*a* and 4*b*). Alewife habitat switches occur between drowned river mouth lakes and Lake Michigan itself during the first season of growth, but individuals originating from drowned river mouth lakes constitute a small fraction of the Lake Michigan population. This could be due to the fact that nearshore and lake-wide habitats of Lake Michigan are significantly more voluminous and would ultimately generate significantly more recruits than drowned river mouth lakes.

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Because individuals of rapidly growing species such as alewife are small, susceptible to dispersive processes, and experience high mortality rates, they are difficult to study by conventional approaches such as mark–recapture studies. Therefore, studies of natural tags such as stable isotope ratios of soft tissues or elemental composition and stable isotope ratios of otoliths represent an alternative. However, only accretionary structures such as otoliths permit recognition a posteriori of habitats occupied throughout the entire life of a fish. Contrary to elemental analysis of otoliths (e.g., Patterson et al. 2004), application of $\delta^{18}O_{oto}$ and $\delta^{13}C_{oto}$ values to fine-scale temporal studies of YOY dynamics has been extremely limited (Thorrold et al. 2001). Most otolith isotope studies used whole crushed otoliths (e.g., Ayvazian et al. 2004) or have recovered relatively low temporal resolution data, resulting in isotope values that integrate the first 6–12 months of a fish's life (e.g., Gao et al. 2001). Such temporal resolution limits potential applications to the early life history of fish.

Our study of alewife otoliths demonstrates that high-resolution sampling can be applied successfully to evaluate the life history of small forage fish otoliths. This technique presents a tremendous opportunity for increasing the temporal resolution of spatial behavior pattern studies. Ultimately, information pertaining to migration and habitat preferences should contribute to the understanding of temporal dynamics and recruitment variability of fish populations.

Acknowledgments

We thank Glenn Carter and Doran Mason of NOAA's Great Lakes Environmental Research Laboratory and the captains and crew of the NOAA Great Lakes Environmental Research Laboratory's RV *Laurentian*, RV *Shenehon*, RV *Cyclops*, and RV *Remorse* for facilitating alewife field collections. We also thank Blaine Novakovski, Christopher Wurster, Tim Prokopiuk, Antoine Zazzo, and Aaron Diefendorf who provided technical assistance in the preparation of otoliths and isotope measurements. This work benefits from the expertise of Patricia Richard and Thierry Bariac (Laboratoire BioMCO, France) in the isotope analysis of water. We also thank Karin Limburg and an anonymous reviewer for their comments. This research was funded by the Great Lake Fisheries Trust (project No. R-00-18).

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The importance of microzooplankton versus phytoplankton to copepod populations during late winter and early spring in Lake Michigan

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Abstract: Feeding rates of the calanoid copepod *Leptodiaptomus sicilis* on natural assemblages of phytoplankton and microzooplankton were evaluated during late winter and early spring in Lake Michigan. Microzooplankton were the preferred food source for this copepod, and larger size fractions of phytoplankton were preferred to smaller size fractions. Ingestion rates of total chlorophyll *a* ranged from 2 to 14 ng·copepod^{-1}·day^{-1}, while ingestion rates of microzooplankton biomass ranged from 0.04 to 0.15 µg C·copepod^{-1}·day^{-1}. In these experiments, microzooplankton carbon accounted for 22%–74% of the total carbon ingested. Clearance rates of microzooplankton carbon were positively related to the larger size fractions of chlorophyll *a* and to total suspended solids. Measured ingestion rates of microzooplankton and phytoplankton carbon suggest that calanoid copepod populations have the potential to control microzooplankton production in late winter and early spring, and even with an abundance of phytoplankton carbon, food availability may limit the reproduction of *L. sicilis*. Because microzooplankton contribute significantly to the diet of these copepods, stimulation of the microbial food web by terrigenous inputs of nutrients and carbon may be transmitted to higher trophic levels (i.e., mesozooplankton and their predators) through heterotrophic flagellates and protozoans.

Résumé : Nous avons déterminé les taux d'alimentation du copépode calanoïde *Leptodiaptomus sicilis* sur les communautés naturelles de phytoplancton et de microzooplancton à la fin de l'hiver et au début du printemps au lac Michigan. Le microzooplancton constitue la source de nourriture préférée de ce copépode et les fractions de tailles plus grandes du phytoplancton sont choisies de préférence aux de tailles plus petites. Les taux d'ingestion de chlorophylle *a* varient de 2 à 14 ng·copépode^{-1}·jour^{-1}, alors que les taux d'ingestion de la biomasse du microzooplancton varient de 0,04 à 0,15 µg C·copépode^{-1}·jour^{-1}. Dans ces expériences, le carbone du microzooplancton représente de 22 à 74 % du carbone total ingéré. Les taux de clearance du carbone du microzooplancton sont en corrélation positive avec les fractions de tailles plus grandes de chlorophylle *a* et avec les solides totaux en suspension. Les taux d'ingestion de microzooplancton et de phytoplancton mesurés laissent croire que les populations de copépodes calanoïdes ont le potentiel de contrôler la production du microzooplancton à la fin de l'hiver et au début du printemps; même s'il y a une abondance de carbone du phytoplancton, la disponibilité de la nourriture peut restreindre la reproduction de *L. silicis*. Parce que le microzooplancton contribue de façon significative au régime alimentaire de ces copépodes, la stimulation du réseau alimentaire microbien par les apports de nutriments et de carbone d'origine terrestre peut se transmettre aux niveaux trophiques supérieurs (c'est-à-dire au mésozooplancton et à ses prédateurs) par l'intermédiaire des flagellés hétérotrophes et les protozoaires.

[Traduit par la Rédaction]

Introduction

Mesozooplankton assemblages of the glacial great lakes of North America, which extend from the Laurentian Great Lakes in the south to Great Bear Lake in the Canadian Arctic, are dominated by calanoid and cyclopoid copepods, especially in winter and early spring (Torke 2001; Barbiero et al. 2001). In Lake Michigan and many of the other lakes, cladocerans are dominant members of zooplankton assemblages in spring and fall (e.g., Torke 1975, 2001; Barbiero et al. 2001). The winter and spring isothermal period is important to calanoids because it is a major reproductive period (e.g., Torke 1975). Calanoids have traditionally been considered to be food-limited in late winter because developmental

Received 21 May 2003. Accepted 31 December 2004. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on 28 September 2005.
J17538

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 doi: 10.1139/F05-111 © 2005 NRC Canada

rates, feeding rates, storage of energy reserves, and reproduction during the spring isothermal period respond positively to increases in phytoplankton biomass and changes in food quality (Vanderploeg et al. 1992*a*). Overwintering females rely heavily on stored lipids for reproduction, and can exist for long periods at extremely low food levels. However, the ability to maintain reproductive output throughout the spring depends on their ability to replenish lipids that are depleted during the initial reproductive effort (Vanderploeg et al. 1992*a*, 1992*b*). Therefore, population success of the copepods that provide food for planktivorous fish during spring and summer is a function of the quantity and quality of food that reproducing females and maturing juveniles encounter in late winter and early spring.

During the late winter – early spring in Lake Michigan, phytoplankton assemblages are dominated by centric diatoms and cryptophytes. These phytoplankton increase in abundance until primary production peaks and then decline as stratification occurs and nutrients are depleted in the upper mixed layer (e.g., Fahnenstiel and Scavia 1987; Barbiero and Tuchman 2001). Although food webs in large lakes have been traditionally been considered to be controlled by autotrophic processes, recent data indicate that, during a large portion of their annual cycle, they may be driven by allochthonous organic inputs and resuspension, rather than in situ primary production (Cotner and Biddanda 2002). Therefore terrigenous organic inputs and associated heterotrophic production can be as important as primary production in the cycling of lake carbon (Grey et al. 2001). The spring diatom bloom has been identified as fuel for the pelagic food web, providing a boost to copepod populations as they begin to feed after overwintering. However, calanoids may enter their peak reproductive period before the spring bloom, when phytoplankton biomass is relatively low (Vanderploeg et al. 1992*a*). Studies conducted over the last two decades suggest that, in large temperate lakes like Lake Michigan, the microbial food web controls carbon transfer during this time (e.g., Carrick et al. 1991; Fahnenstiel et al. 1998; Biddanda and Cotner 2002).

Because heterotrophic protists can provide an important link between microbial food webs and higher trophic levels (e.g., Carrick et al. 1991; Lampman and Makarewicz 1999), information about mesozooplankton feeding on autotrophic versus heterotrophic components of the seston is critical to understanding pathways of energy transfer and the potential contribution of the microbial food web to productivity at higher trophic levels. There is a shortage of information about the relative contribution of microzooplankton versus phytoplankton to copepod diets in large temperate lakes, and little is known about the influence of different phytoplankton size fractions and taxa on copepod consumption of microzooplankton (Burns and Gilbert 1993). It is also unclear whether or not copepod selective feeding can control rates and pathways of carbon transfer by removing a significant amount of either phytoplankton or microzooplankton biomass.

This work was carried out as part of the National Science Foundation – National Oceanic and Atmospheric Administration Episodic Events – Great Lakes Experiment (NSF – NOAA EEGLE), and experiments were conducted simultaneously with EEGLE oceanographic survey cruises along established transects running perpendicular to the shore in southern Lake Michigan (Fig. 1). The goal of the EEGLE project was to investigate effects on the ecology of Lake Michigan of late winter – early spring storm events that resuspend sediment particles and associated nutrients and toxins (Eadie et al. 1996; Robbins and Eadie 1991). By stimulating heterotrophic production in nearshore regions of the lake, episodic physical processes, such as resuspension or increased runoff that locally increase terrigenous inputs of carbon and nutrients, can be important to the production of higher trophic levels. The pathways for this increased production may move energy from the microbial food web through copepods to larval and adult planktivorous fish.

To reach a better understanding of the potential for resuspension events to affect the lower levels of the pelagic food web, we conducted copepod feeding experiments in 1998–2000 using natural Lake Michigan seston. Here we focus on the feeding responses of the calanoid *Leptodiaptomus sicilis* because this copepod is an omnivore that occurs in all of the Great Lakes, it is one of the dominant calanoids in Lake Michigan during late spring – early winter (Robertson 1966; Barbiero et al. 2001), and it is a primary prey item for planktivorous fish (e.g., Evans 1990). This species is well adapted to low temperatures. One of the authors (H.A. Vanderploeg) has examined feeding and reproduction of *L. sicilis* in a number of environments during the winter at Great Lakes sites, as well as under ice cover at Great Slave Lake in the Canadian subarctic. These copepods feed and reproduce at temperatures just above 0 °C at very low particle concentrations. Our goal was to investigate the potential for copepods to utilize microzooplankton and phytoplankton biomass in natural plankton communities during late winter and early spring, and to examine the possibility that copepod populations can control microzooplankton production during this physically dynamic time when storm-induced resuspension events frequently occur.

Methods

Water, zooplankton for experiments, physical and biochemical data, and zooplankton abundance data were collected from stations along EEGLE survey transects (Fig. 1; Table 1). All experiments were conducted between February and April before water column thermal stratification occurred. At each station, conductivity, temperature, and depth were measured using a Sea-Bird Electronics Sealogger Profiler (model No. SBE 25; Sea-Bird Electronics, Inc. Bellevue, Washington, USA). Dry weight of total suspended solids (TSS) was determined gravimetrically from discrete water samples collected at each experimental site.

Zooplankton were collected using a large aspect ratio (0.5 m diameter, 2.25 m length) 202- or 303-μm mesh plankton net with a solid cod end that was slowly towed vertically to the surface from 10 m above the bottom. Water for experiments was collected 10 m below the surface using a 30-L Niskin bottle that was slowly and gently emptied into polycarbonate carboys through a submerged hose. In some cases, water was gently screened through a submerged 400-μm mesh net to remove stray metazoans such as

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Fig. 1. Location of stations (□) and transects (solid lines) of National Science Foundation – National Oceanic and Atmospheric Administration Episodic Events – Great Lakes Experiment survey cruises in Lake Michigan. Dotted lines, depth contour internals (10 m). Square in inset shows location of the study area in the Great Lakes region.

copepods and copepodites, while in other cases, metazoans were removed by pipetting.

Zooplankton were maintained at close to ambient temperatures in insulated containers until grazing experiments commenced. Adult female cephalothorax length, body volume, and dry weight are approximately 1.2 mm, 1.0 μL, and 30 μg, respectively (Vanderploeg et al. 1992*b*). Carbon content is approximately 45% for calanoids (Mauchline 1998). Grazing experiments were conducted at ambient temperatures under dim light and a 12 h light – 12 h dark regime. Nine 1180- or 610-mL Pyrex bottles were slowly filled with water from the site, and non-gravid *L. sicilis* females were individually pipetted into four of the bottles for a final concentration of 17–34 copepods·L^{-1} (mean concentration was 29.4 copepods·L^{-1}). Controls consisted of three bottles that were identical to experimental bottles but without added copepods. Three initial bottles were treated the same as the controls were, but were sampled for phytoplankton and microzooplankton approximately 1 h after all the bottles were filled. Bottles were sealed without bubbles and placed on a plankton wheel that rotated at 0.25 revolutions·min^{-1}. Nutrients were not augmented in these experiments because a preliminary experiment that compared final chlorophyll *a* (Chl *a*) concentrations in grazing bottles with and without added nutrients indicated that zooplankton excretion was not a significant factor influencing phytoplankton growth. An isotope-addition experiment confirmed these results (W.S. Gardner, The University of Texas at Austin, Marine Science Institute, 750 Channel View Drive, Port Aransas, TX 78373-5015, USA, personal communication). After 24 h, copepods were pipetted from grazing bottles and samples were collected from each control and grazing bottle for size-fractionated Chl *a* (Bowers 1980; Vanderploeg et al. 2001) and microzooplankton enumeration. The 1998 and February

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Table 1. Station data.

Date	Station	TSS (mg·L^{-1})	Temperature (°C)	Chl *a* size fraction	Chl *a* (µg·L^{-1})	Phytoplankton carbon > 5 µm from cell counts (µg·L^{-1})	Picophytoplankton carbon (µg·L^{-1})	Phytoplankton carbon : Chl *a*	Initial microzooplankton carbon (µg·L^{-1})
1 April 1998	M110	1.70	4.0	>53	0.28	14.0	n/a	n/a	2.0
				>10–53	0.26				
				<10	0.73				
				Total	1.27				
25 February 1999	M45	1.00	1.5	>53	0.19	n/a	9.5	n/a	9.1
				>10–53	0.13				
				<10	0.78				
				Total	1.10				
13 March 1999	J60	0.60	1.0	>53	0.27	n/a	21.2	n/a	8.6
				>10–53	0.12				
				<10	0.93				
				Total	1.32				
7 April 1999	C80	1.80	3.0	>53	0.44	14.0	9.0	13.1	8.8
				>10–53	0.24				
				<10	1.08				
				Total	1.75				
20 February 2000	J80	1.10	2.0	>53	0.45	8.5	15.4	19.0	6.9
				>10–53	0.21				
				<10	0.60				
				Total	1.26				
22 February 2000	G45	1.00	2.0	>53	0.35	8.5	19.2	22.9	6.9
				>10–53	0.22				
				<10	0.87				
				Total	1.96				
11 March 2000	J30	3.00	2.0	>53	0.50	13.7	11.7	21.2	8.7
				>10–53	0.13				
				<10	0.57				
				Total	1.20				
12 March 2000	NB20	4.40	3.0	>53	0.71	45.4	3.3	24.4	5.6
				>10–53	0.28				
				<10	1.01				
				Total	2.00				
13 March 2000	C80	1.10	2.0	>53	0.68	13.3	8.4	11.9	5.6
				>10–53	0.25				
				<10	0.89				
				Total	1.82				

Note: TSS, total suspended solids; n/a, not applicable.

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and March 1999 experiments were conducted in walk-in environmental rooms at the NOAA Great Lakes Environmental Research Laboratory (GLERL) with water and zooplankton that were collected within 24 h. All other experiments were conducted in incubators on shipboard.

For microzooplankton samples, initial and final 100-mL samples were removed from each bottle, preserved in 1% acid Lugol's iodine, and stored at 4 °C until they were counted. Triplicate 50- to 100-mL subsamples were settled in an Utermöhl chamber (Utermöhl 1958), and the entire chamber was then counted under an Olympus IX-70 DIC (differential interference contrast) inverted microscope. To estimate microzooplankton biomass, the linear dimensions of 30–90 individuals (fewer for less abundant taxa) were measured at 400×–1000× and converted to volumes using the appropriate geometric shapes. The volume of tintinnid cells was determined using the same approach as that used for aloricate protists, since they were clearly visible inside their loricas under differential interference contrast microscopy.

To estimate microzooplankton biomass in grazing experiments, we used the volume-to-carbon regression of Putt and Stoecker (1989) for ciliates. Their data were obtained using the same fixative as that used in this study (Lugol's iodine), as well as ciliate species that were similar to the predominant taxa in Lake Michigan. The regression includes a correction of about 20% for cell shrinkage due to preservation. Recent studies indicate that some ciliates can shrink by as much as 40% in Lugol's iodine (e.g., Chaput and Carrias 2002). Therefore our estimates of ciliate carbon may be conservative. For dinoflagellates, we used the equations from Menden-Deuer and Lessard (2000) to convert volume to carbon. Such equations have been shown to be more appropriate for estimating freshwater plankton biomass than those considering all protist groups as a whole (Gosselain et al. 2000). These dinoflagellate volume estimates were corrected assuming 30% shrinkage (Montagnes et al. 1994).

During the April 1999 experiment and all of the 2000 experiments, samples were taken from initial bottles and preserved in Lugol's solution so that the abundance and species composition of ambient phytoplankton assemblages could be related to clearance rates of Chl *a* size fractions. For the April 1999 experiment, phytoplankton samples were collected at the site 2 days prior to the experiment. Subsamples (10- to 50-mL) were filtered onto membrane filters for permanent mounting on slides. Phytoplankton >5 µm were identified to the lowest practical taxonomic unit (generally to species or genus). Phytoplankton were also classified by broad taxonomic group (i.e., diatoms, flagellates, cryptophytes, chlorophytes, chrysophytes, and cyanobacteria) and by biovolume: cells that were >1000, 1000–200, and <200 μm^3 were designated as large, medium, and small, respectively.

Photosynthetic picoplankton (in this case, cells <3 µm, which were mostly single-celled *Synechococcus*-like cyanobacteria) were preserved with 2% paraformaldehyde, filtered onto 0.2-µm pore size black polycarbonate membrane filters, and frozen at –20 °C immediately after collection until counted via epifluorescence microscopy (MacIsaac and Stockner 1993). Picoplankton cells were sized using Image Pro 4.5 image analysis software and a SPOT-2 digital camera. At least 500 cells were measured in each sample. Cell volumes were converted to carbon based on a conversion factor for cold-water picoplankton of 0.21 pg C μm^{-3} (Booth et al. 1993).

Grazing rates on phytoplankton were determined by measuring the disappearance of chlorophyll size fractions (>53, 10–53, and <10 µm) in experimental bottles. Using the disappearance of chlorophyll to measure grazing is a common method that provides results similar to short-term ingestion experiments using radiolabeled algae (Hargis 1977). Strictly speaking, the method measures assimilation, and problems could arise with digestive-resistant algae; however, digestive-resistant algae were not part of the winter or spring algal assemblage that was examined here. Excluding phaeopigments from the analyses reduced the potential for fluorescence from pigments in fecal material to bias calculations of ingestion and clearance rates. The use of size-fraction analyses also provided a means of determining feeding and selectivity (measured as relative clearance rates, sensu Vanderploeg 1994) of different size classes of algae.

Size-fractionated Chl *a* was analyzed by filtering two replicate subsamples (usually 180 mL) of water from initial, control, and grazing bottles through an apparatus containing a set of stacked Nitex screens and a GF/F filter immersed in water (Bowers 1980; Vanderploeg et al. 2001). The largest phytoplankton fraction was collected on a 53-µm mesh screen followed by a 10-µm mesh screen, and then a GF/F filter (nominal pore size, 0.7 µm). Little flocculation was noted in the jars, so retention of smaller cells on the larger meshes most likely did not bias our measurements (Logan et al. 1994). Screens and filters were folded and inserted into plastic test tubes, then frozen and later extracted with *N,N*-dimethylformamide and analyzed fluorometrically (Speziale et al. 1984). Total Chl *a* was obtained from the sum of the three size fractions.

In experiments like these, there is always the risk of preferred prey being removed to a greater degree than other prey, thus altering the relative concentrations over the experimental period. Concentrations of Chl *a* were always greater than 44% of original concentrations for all size fractions in all experiments except the 12 March 2000 experiment, in which the 10–53 µm fraction fell to 34% of the original concentration. Microzooplankton were severely depleted in some cases. In some studies, this relative depletion of preferred prey resulted in the underestimation of clearance rates of phytoplankton and microzooplankton carbon (e.g., Zeldis et al. 2002). However, for *L. sicilis*, unless a prey is completely eliminated, it is unlikely that changing relative prey concentrations will greatly affect clearance rates, and therefore copepod selectivity, if concentrations of prey are initially below the incipient limiting concentration. Feeding experiments similar to these that used large and small phytoplankton prey in bottle experiments show that selectivity of *L. sicilis* does not change over a broad range of relative concentrations (Vanderploeg et al. 1984). Selectivity and feeding experiments conducted with long- and short-term exposure of diaptomids to algae with radiolabeled carbon and phosphorus (Demott 1990; Demott 1995) showed that, in situations in which there was depletion of preferred prey over longer incubations, long- and short-term experiments provided much the same results, with no evidence that short-term exposure provided higher clearance rates.

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Clearance rates and ingestion rates of each size fraction of Chl *a* were calculated using Frost's equations (Frost 1972). Copepod ingestion and clearance rates when feeding on microzooplankton were calculated from initial and final microzooplankton biomass according to Gifford (1993). So that we could make general comparisons between this and other published studies and relate the autotrophic biomass to heterotrophic biomass that was consumed by copepods during this study, Chl *a* concentrations in each size fraction were converted to carbon using a carbon : Chl *a* ratio of 20. This ratio was derived from biomass estimates from >5-μm cell counts that were made during the April 1999 and March–April 2000 experiments, which were compared with Chl *a* in the total size fraction. Cell dimensions of different taxa were converted to cell volume and then to carbon from estimates derived from empirical studies for diatoms (Strathmann 1967) and non-diatoms (Verity et al. 1992). Conversion equations from these studies used non-linear regression models to account for higher carbon content per unit volume in smaller cells, and experiments included a wide range of cell sizes. These two models produce similar results to regression models derived from experimental data combined with data from literature studies (Menden-Deuer and Lessard 2000). However, the model of Strathmann (1967) may underestimate carbon by 20% for diatoms less than 30 μm^3 in biovolume and may overestimate carbon by 5%–60% as diatoms increase in biovolume from 300–10^6 μm^3 (Menden-Deuer and Lessard 2000). The dominant diatom species in the present study were found in the medium and small size classes, in which biovolumes were in the range of 20–800 μm^3. Therefore, algal carbon contained in the larger size fractions may have been overestimated when large diatoms dominated the phytoplankton community. By including the biomass of picocyanobacteria at each station (which ranged from 3.3 to 21.2 μg $C \cdot L^{-1}$; Table 1), an average ratio of 18.8 for all experiments was obtained. For our general comparisons, we rounded the ratio to 20. Our empirically derived carbon : Chl *a* ratio approximates the ratio that can be derived from data collected at similar temperatures in an earlier study in Lake Michigan in early spring (Fahnenstiel and Scavia 1987, their fig. 2), and is also within the range empirically derived for estuarine phytoplankton (Gallegos and Vant 1996).

In addition to directly affecting phytoplankton concentrations in experimental bottles through grazing, mesozooplankton indirectly affect phytoplankton concentrations by feeding on microzooplankton grazers and thereby reducing this component of mortality (Nejstgaard et al. 2001). During the 2000 experiments, dilution experiments (Landry 1993) were conducted to assess the grazing impact of microzooplankton on phytoplankton assemblages. Water used in dilution experiments was collected at the same time as water used in copepod grazing experiments. The size fractions examined in dilution experiments were 25, 5, and 0.4 μm. The correction factor (k_p) for the effects of copepod predation on microzooplankton grazers, was derived according to the methods of Nejstgaard et al. (2001):

$$(1) \quad g_{corr,p} = g_{cop,p} + k_p$$

$$(2) \quad k_p = g_{mic,p}(\hat{c} - \hat{c}^*)\hat{c}^{-1}$$

$$(3) \quad \hat{c} = (c_t - c_0)(\ln(c_t/c_0))^{-1}$$

$$(4) \quad \hat{c}^* = (c_t^* - c_0)(\ln(c_t^*/c_0))^{-1}$$

where $g_{corr,p}$ is the corrected copepod grazing coefficient on a specific size fraction p; $g_{cop,p}$ is the uncorrected copepod grazing coefficient; $g_{mic,p}$ is the microzooplankton grazing coefficient determined from dilution experiments; $\hat{c}$ and $\hat{c}^*$ are microzooplankton biomass in the control and grazing bottles, respectively, at the end of the experiment; c_t is microzooplankton biomass at time t; and c_0 is the initial microzooplankton biomass.

For the 1998 and 1999 grazing experiments, in which concurrent dilution experiments were not conducted, the average value from the 2000 experiments was used as an estimate of $g_{mic,p}$. In all experiments, the g_{mic} determined for the <25-μm fraction of Chl *a* in dilution experiments was used to correct copepod grazing on the <53-μm fraction. Because more than 85% of the microzooplankton present during experiments were less than 60 μm in the longest dimension, no correction was used for the >53-μm fraction because it was assumed that the highest feeding rates of the majority of microzooplankton were primarily for phytoplankton cells smaller than their longest dimension.

Statistics

To test for the effect of microzooplankton biomass and each size fraction of Chl *a* on copepod clearance rates, each station–date combination was treated as an independent sample ($n = 9$, with three or four replicates at each size fraction for a clearance rate of Chl *a*, and three or four replicates of clearance rate of microzooplankton biomass per experiment; Table 1). Levine's test showed that clearance rates were significantly heteroscedastic with respect to date. Therefore, we log-transformed clearance rate data and conducted an analysis of variance (ANOVA; SAS Version 7: Proc GLM; Potvin and Roff 1993) to examine the direct effects of food category (i.e., the three size fractions of Chl *a* and microzooplankton carbon) and sample date on copepod clearance rate. Type-III sum of squared error was used for the interpretation of results. Unless otherwise noted, a posteriori comparisons were made using the Newman–Keuls procedure (SNK test).

Spearman correlation analyses were also used to explore individual relationships among the following: (*i*) clearance rates of each size fraction of Chl *a* with TSS, temperature, initial Chl *a* in each size fraction, initial microzooplankton carbon, and clearance rates of microzooplankton carbon; (*ii*) clearance rates of microzooplankton carbon with TSS, temperature, clearance rates of total Chl *a*, initial microzooplankton carbon, and initial Chl *a* concentration; (*iii*) initial concentrations of total Chl *a* with initial microzooplankton carbon, TSS, and temperature; and (*iv*) initial microzooplankton carbon with TSS and temperature. A sequential Bonferroni-type procedure was used to control the false-discovery rate for independent statistics, as described in Benjamini and Hochberg (1995).

Results

Total initial phytoplankton biomass, estimated from the sum of initial carbon in each size fraction, ranged from 22.0

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Fig. 2. Initial microzooplankton and phytoplankton biomass (μg C·L^{-1}) for each experiment. Shaded bars, microzooplankton; solid bars, phytoplankton.

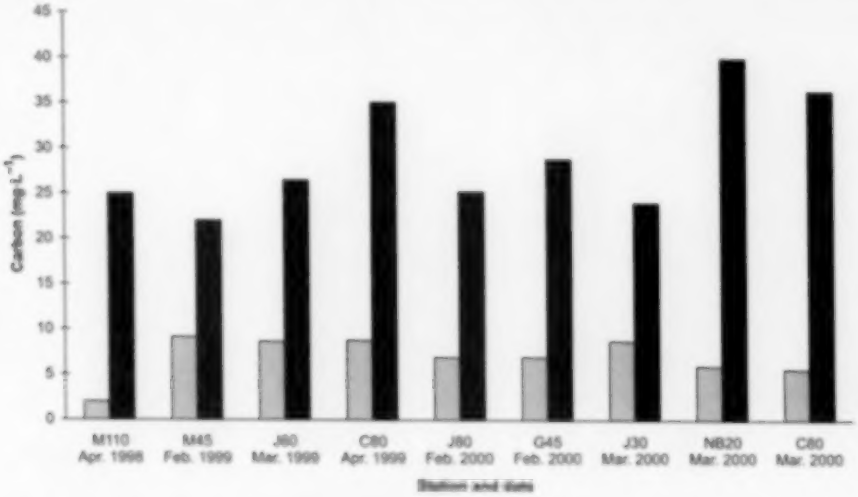
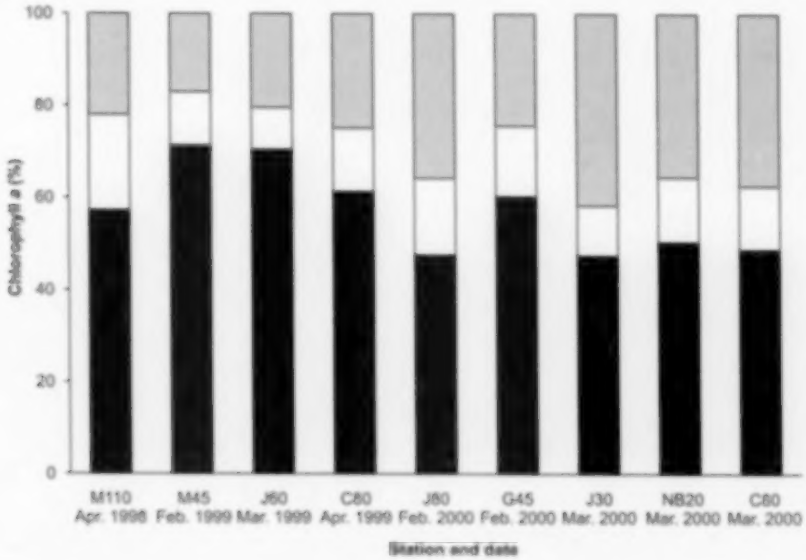


Fig. 3. Size distributions of initial chlorophyll *a* for each experiment, as percentage of total Chl *a*. Shaded bars, >53 μm; open bars, 10–53 μm; solid bars, <10 μm.



to 40.0 μg C·L^{-1}, while initial microzooplankton biomass ranged from 2.0 to 9.1 μg C·L^{-1} (Table 1; Fig. 2). When data from all experiments were pooled, the <10-μm fraction was the largest fraction of initial Chl *a* in all experiments (ANOVA, $p < 0.001$; SNK a posteriori test, $p < 0.05$), making up more than 50% of Chl *a*, and there were no significant differences in Chl *a* concentration between the 10–53 and the >53-μm fractions. When experiments were considered separately, the <10-μm fraction was always qualitatively the largest fraction of Chl *a* (more than 50% of the total), and the >53-μm fraction was larger than the 10–53 μm fraction (Fig. 3). Biomass (carbon) was also dominated by phytoplankton (Fig. 2).

The available data from microscopic counts of >5-μm phytoplankton showed that the <10-μm fraction, which was the largest fraction of Chl *a* in each of these experiments (Fig. 3), was numerically dominated by nanoflagellates, cyanobacteria, small diatoms, and chlorophytes, although species composition varied among experiments. Diatoms and cryptophytes composed most of the 10–53 μm fraction, and diatoms composed the >53-μm fraction.

The size fractions that accounted for most of the biomass varied among experiments (Fig. 4), as did the dominant taxa. In contrast with other stations where small cells accounted for most of the biomass, the dominant species at NB20 and C80 during March 2000 was the cryptophyte *Cryptomonas*

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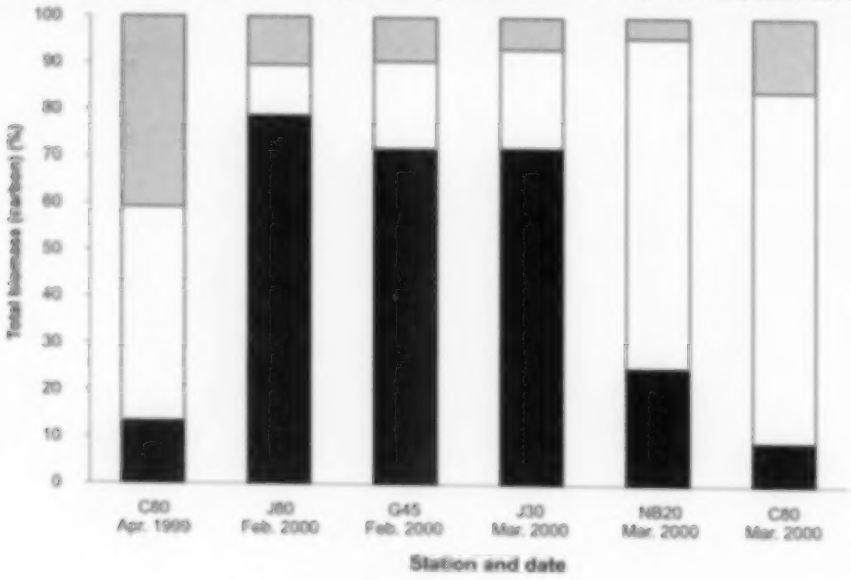
Fig. 4. Taxonomic composition of initial phytoplankton assemblage for each experiment in which cell counts were conducted, shown as a percentage of total biomass (carbon). Shaded bars, large cells; open bars, medium cells; solid bars, small cells.



erosa (~1800 μm^3), which made up approximately 60% of the biomass. At C80 in April 1999, single-celled (*Synedra ulna*) and chain-forming and colonial diatoms (e.g., *Tabellaria fenestra* and *Melosira italica*) were the other dominant taxa in the >53-µm fraction in most other experiments. In the March 2000 J30 experiment, the chlorophyte *Gloeocystis planktonica* (~100 μm^3) was abundant (~28% abundance and 24% biomass). Picophytoplankton (mostly *Synechococcus*-like cyanobacteria) were abundant at all stations, and their biomass ranged from 3.3 to 21.2 µg $C \cdot L^{-1}$ (Table 1). The dominant microzooplankton taxa were ciliates (tintinnids, oligotrichs, prostomatids, and scuticociliates), with most cells in the range of 1500 – 60 000 μm^3, and a heterotrophic dinoflagellate, *Gymnodinium helveticum* (19 600 μm^3). Direct counts to quantify changes in microzooplankton species distributions during feeding experiments showed that oligotrichs and tintinnids in the 30- to 50-µm equivalent spherical diameter (ESD) range were most vulnerable to copepod predation (Kovalcik 2001).

When experiments were pooled, copepod clearance rate of microzooplankton carbon was significantly higher than the clearance rate of any size fraction of Chl *a* (ANOVA on rank-transformed data, $p < 0.0001$; SNK a posteriori test, $p < 0.05$; Fig. 5*a*). Clearance rate of the 10–53 µm fraction was significantly higher than clearance rate of the >53-µm and the <10-µm fraction (SNK a posteriori test, $p < 0.05$; Fig. 5*a*).

Clearance rates of microzooplankton carbon ranged from 14.3 (M45, February 1999) to 37.0 mL·copepod^{-1}·day^{-1} (NB20, March 2000; Table 2). When the experiments were considered separately, clearance rates of copepods feeding on microzooplankton were always among the highest of all food categories, and clearance rates of the 10–53 µm fraction were always equivalent or next highest (Table 3; Fig. 5*b*). Clearance rates of the preferred size fraction of Chl *a* (i.e., the fraction for which clearance rates were highest; Vanderploeg et al. 1984) ranged from 7.0 (C80, April 1999) to 13.4 mL·copepod^{-1}·day^{-1} (NB20, March 2000). At the end of the experiments, concentrations of the most preferred phytoplankton size fraction (10–53 µm) averaged 62% of initial concentrations, while the concentrations of the <10-µm and >53-µm size fractions averaged 80% and 83%, respectively. In some instances, microzooplankton prey were depleted from some of the experiments, and the concentration at the end of the experiments was, on average, 22% of initial concentrations.

Ingestion rate total Chl *a* ranged from 2 (J30, March 2000) to 14 ng·copepod^{-1}·day^{-1} (NB20, February 2000) or from 40 to 28 ng C·copepod^{-1}·day^{-1}, respectively (Table 2). Ingestion rates of microzooplankton biomass ranged from 0.04 µg (M110, April 1998) to 0.15 µg C·copepod^{-1}·day^{-1} (C80, April 1999; Table 2). Microzooplankton carbon accounted for between 22% and 74% of the carbon ingested (i.e., total phytoplankton carbon ingested + microzooplankton carbon ingested) across all experiments (Fig. 6).

There was a significant positive relationship ($p < 0.05$) between clearance rates of microzooplankton carbon and initial concentrations of Chl *a* in the 10–53 µm fraction (Spearman's rho = 0.70, $n = 9$), TSS (Spearman's rho = 0.82, $n = 9$), and temperature (Spearman's rho = 0.76, $n = 9$). There was also a significant relationship between clearance rates of the >53-µm size fraction of Chl *a* and temperature (Spearman's rho = 0.80, $n = 9$).

There were no significant relationships between clearance rates of any other size fraction of Chl *a* and TSS, temperature, initial Chl *a* in any size fraction, initial microzooplankton carbon, or clearance rates of microzooplankton carbon. There were no significant relationships between ingestion rates of total Chl *a* and TSS or temperature. There were also no significant relationships between initial Chl *a* concentra-

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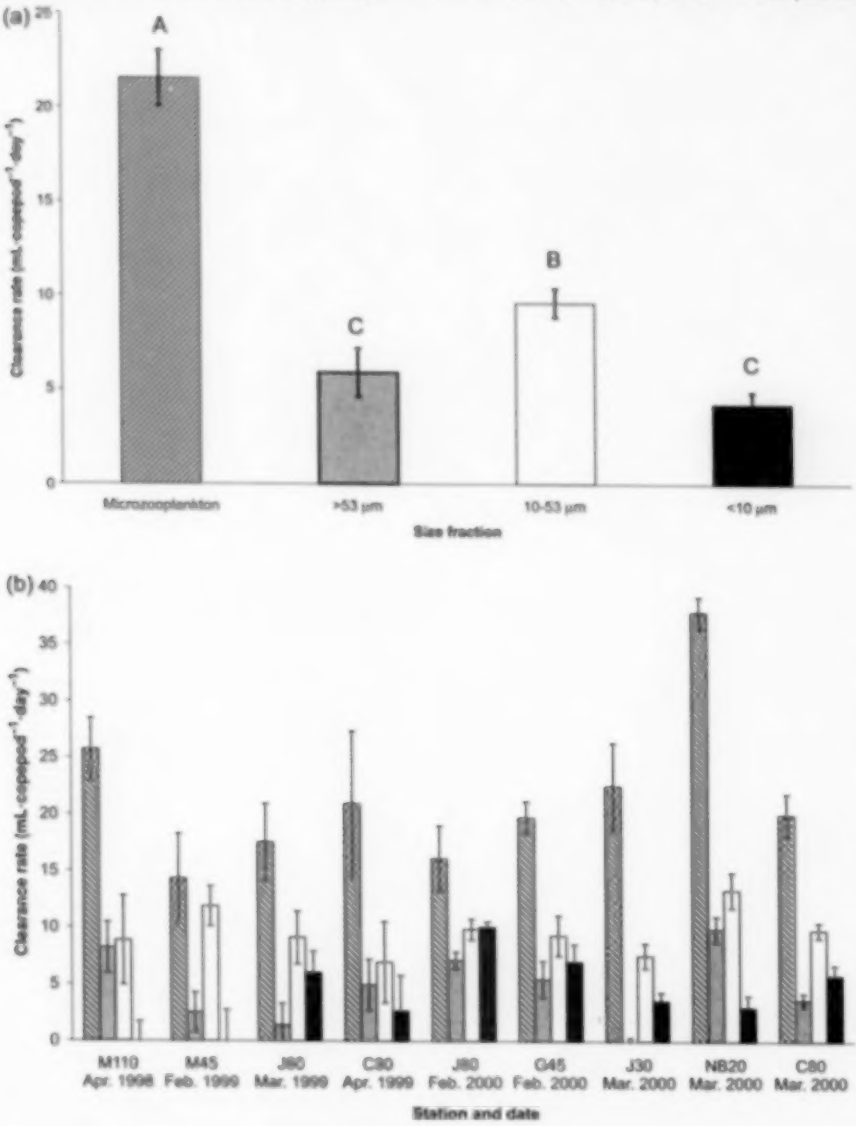
Fig. 5. Clearance rates (mL·copepod^{-1}·day^{-1} ± standard error) of microzooplankton and Chl *a* size fractions. (*a*) All experiments considered together. Letters (A > B > C) indicate ranking of clearance rates (analysis of variance, $p < 0.0001$; Newman–Keuls procedure, $p < 0.05$). (*b*) Individual experiments. Hatched bars, microzooplankton; shaded bars, >53 µm; open bars, 10–53 µm; solid bars, <10 µm.



tion or initial microzooplankton biomass and TSS or temperature, or between TSS and temperature ($p > 0.05$ for all non-significant comparisons).

Discussion

Selective feeding

Leptodiaptomus sicilis, like many calanoids, is a highly selective feeder, and feeding rates on prey that are not the preferred size or shape can be an order of magnitude lower than those on preferred prey (Vanderploeg et al. 1988; Vanderploeg 1994). In general, *L. sicilis* selects prey that are larger than 10 µm ESD (Vanderploeg et al. 1992*a*). The smallest particles *L. sicilis* typically feeds upon are approximately 3 µm ESD, which are captured passively and without remote detection (Vanderploeg and Paffenhöfer 1985). The diameter of the mouth most likely limits ingestion of whole cells to those less than 30 µm in diameter. Feeding preferences for larger (>10 µm) phytoplankton have been observed in natural seston (Bowers 1980; Vanderploeg et al. 1984), and rates as high as 41.5 mL·copepod^{-1}·day^{-1} have been observed for diaptomids feeding on elongated diatoms (~100 µm in longest dimension; Vanderploeg et al. 1988). In the present study, the highest clearance rates on phytoplankton were observed for the 10–53 µm size fraction. Large- and mid-sized diatoms that are elongated in one di-

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Table 2. Clearance rates and ingestion rates.

Date	Station	Chl *a* size fraction	Chl *a* clearance rate (mean ± SE; mL·copepod·day^{-1})	Chl *a* ingestion rate (ng·copepod^{-1}·day^{-1})	Phytoplankton carbon ingestion rate (µg·copepod·day^{-1})	Microzooplankton carbon clearance rate (mean ± SE; mL·copepod·day^{-1})	Microzooplankton carbon ingestion rate (µg·copepod·day^{-1})
1 April 1998	M110	>53	8.20±1.8	2.30	0.05	25.4±4.0	0.04
		>10–53	8.86±1.8	2.33	0.05		
		<10	0.00	0.00	0.00		
		Total		4.63	0.09		
25 February 1999	M45	>53	2.52±1.9	0.47	0.01	14.3±3.4	0.12
		>10–53	11.88±2.3	1.51	0.03		
		<10	0.00	0.00	0.00		
		Total		1.98	0.04		
13 March 1999	J60	>53	3.76±2.3	1.02	0.02	17.5±6.4	0.13
		>10–53	9.11±3.6	1.09	0.02		
		<10	6.01±3.1	5.61	0.11		
		Total		7.72	0.15		
7 April 1999	C80	>53	4.94±0.8	2.15	0.04	20.9±2.9	0.15
		>10–53	6.95±0.9	1.68	0.03		
		<10	2.66±0.5	2.86	0.06		
		Total		6.69	0.13		
20 February 2000	J80	>53	7.11±1.6	3.20	0.06	16.1±1.4	0.09
		>10–53	9.85±1.8	2.07	0.04		
		<10	10.05±1.6	6.03	0.12		
		Total		11.30	0.23		
22 February 2000	G45	>53	0.30±0.3	0.11	0.00	19.7±3.8	0.12
		>10–53	9.29±1.1	2.04	0.04		
		<10	7.09±0.8	6.17	0.12		
		Total		8.32	0.17		
11 March 2000	J30	>53	–0.53±1.2	0.15	0.00	22.5±1.4	0.14
		>10–53	7.52±1.6	0.98	0.02		
		<10	3.56±1.1	2.03	0.04		
		Total		3.16	0.06		
12 March 2000	NB20	>53	9.88±0.6	7.01	0.14	37.0±1.9	0.14
		>10–53	13.36±0.7	3.74	0.07		
		<10	2.96±0.9	2.99	0.06		
		Total		13.75	0.27		
13 March 2000	C80	>53	3.67±0.3	2.50	0.05	20.0±2.6	0.09
		>10–53	9.80±0.9	2.45	0.05		
		<10	5.80±0.5	5.16	0.10		
		Total		10.11	0.20		

Note: SE, standard error.

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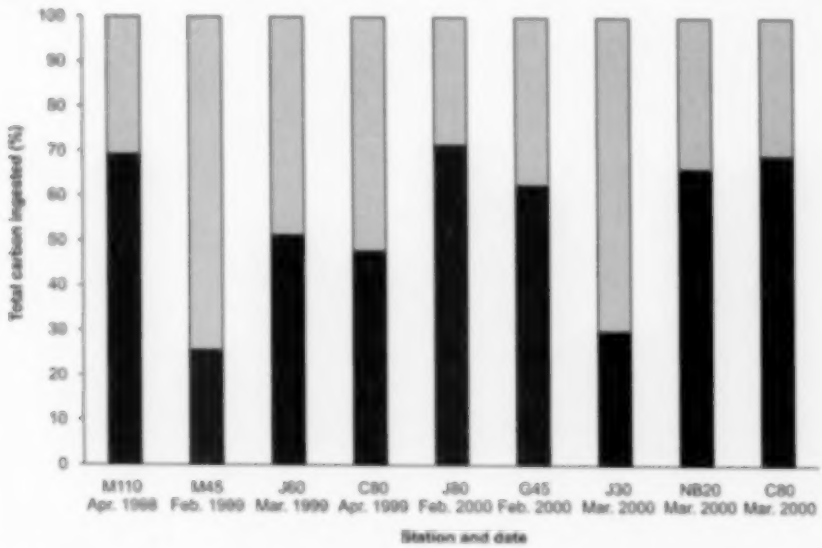
Table 3. Analysis of variance (ANOVA) results for each experiment.

Date	Station	Microzooplankton	Chl *a* size fraction >53 µm	10–53 µm	<10 µm	*p* value
1 April 1998	M110	A	B	B	C	0.0001
25 February 1999	M45	A	B	A	B	0.001
13 March 1999	J60	ns	ns	ns	ns	ns
7 April 1999	C80	A	B	B	C	0.0001
20 February 2000	J80	A	B	AB	AB	0.05
22 February 2000	G45	A	C	B	B	0.0001
11 March 2000	J30	A	D	B	C	0.0001
12 March 2000	NB20	A	C	B	D	0.0001
13 March 2000	C80	A	D	B	C	0.0001
All experiments		A	C	B	C	0.0001

Note: Letters indicate ranking (A > B > C) from Newman–Keuls procedure. Shading indicates size fraction that resulted in lowest clearance rates. ns, not significant.

Fig. 6. Percentage of carbon ingested as microzooplankton versus phytoplankton for each experiment. Shaded bars, microzooplankton; solid bars, phytoplankton.



mension dominated this fraction. Even though microzooplankton biomass was much lower than phytoplankton biomass at all stations, clearance rates of microzooplankton carbon were always higher. In the present study, microzooplankton were, in some cases, completely depleted in the experimental containers. While this depletion was not anticipated or planned, it supports our conclusions that microzooplankton are preferred over phytoplankton as prey.

The feeding preferences for larger (30–50 µm ESD) microzooplankton prey that we observed may stem from an increase in the copepod's ability to detect larger, more motile prey at greater distances than similar-sized, non-motile prey (e.g., Williamson and Vanderploeg 1988). Numerical models and visual observations indicate that particles entrained in the copepod feeding current produce a disturbance downstream that can be detected by copepod sensory structures (Bundy and Vanderploeg 2002; Visser and Jonsson 2000), and *L. sicilis* is capable of detecting large spherical prey when they are more than a copepod body length away (Bundy et al. 1998). The preferences for larger phytoplankton cells (in this case, mostly elongated diatoms and flagellates) and microzooplankton may therefore arise because motile prey and larger elongated cells produce a larger hydromechanical disturbance than small, non-motile cells (Vanderploeg et al. 1984). The chemosensory ability of *L. sicilis*, in terms of detecting phytoplankton cells that are remotely located, has not been well described. However, chemosensory-mediated selection that occurs after a particle contacts the mouthparts (i.e., taste) does play a role in controlling feeding preferences of this copepod.

Top-down control of microzooplankton

When coupled with data on ambient microzooplankton biomass, our results suggest that copepods have the potential to control microzooplankton populations in Lake Michigan during late winter – early spring. With an initial microzooplankton biomass ranging from 2.0 to 9.1 µg C·L^{-1} and an abundance of approximately 3 adult diaptomid copepods·L^{-1}

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during the late winter – early spring cruises (Agy 2001), copepod ingestion of microzooplankton could potentially remove between 1% and 6% of the standing crop of microzooplankton at the lowest ingestion rates observed in these experiments (0.04 μg C·copepod^{-1}·day^{-1}). At the highest ingestion rates observed (0.15 μg C·copepod^{-1}·day^{-1}), copepods could potentially remove between 5% and 23% of microzooplankton standing stock per day. These ingestion rates are conservative because they are calculated using time-averaged, rather than initial (i.e., ambient), prey concentrations in the experimental containers, and they are within the ranges observed for other small copepods in marine and estuarine systems (e.g., Gifford and Dagg 1988; Tiselius 1989). Assuming that microzooplankton production during the spring ranges from 1 to 5 μg C·L^{-1} (Carrick et al. 1992; Kovalcik 2001), adult diaptomids could potentially remove as much as 45% of new microzooplankton production per day. These conclusions are supported by studies in other freshwater systems showing that selective feeding by calanoid copepods can significantly alter microzooplankton biomass and community structure (e.g., Wiackowski et al. 1994; Jack and Gilbert 1997; Adrian and Schneider-Olt 1999).

The above estimates of copepod predation pressure on microzooplankton populations do not consider the grazing impact of diaptomid juveniles and nauplii. For example, Burns and Gilbert (1993) found that clearance rates of diaptomid juveniles feeding on microzooplankton were approximately 35% of adult clearance rates, and that, in contrast with adults, clearance rates were higher for copepods feeding on smaller (<10 μm in the longest dimension) ciliates than for larger (>16 μm) ciliates. Because of their high abundance, clearance rates of ciliates by diaptomid juveniles can equal adult clearance rates (Adrian and Schneider-Olt 1999). Abundances of diaptomid nauplii and copepodites in spring in Lake Michigan are on the order of 5 and 2.5·L^{-1}, respectively (Torke 1975; Barbiero et al. 2001), so an estimate of the grazing pressure of the entire calanoid community, including juveniles, could be in the range of 2%–43% of the standing stock and they could potentially remove as much as 80% of new microzooplankton production. It is important to note that with the inclusion of nauplii and copepodites, a wide size range of microzooplankton would be impacted.

Contribution of microbial food web to copepod production

In most freshwater and marine pelagic systems, the major consumers of picoplankton are heterotrophic flagellates and protozoans (e.g., Porter et al. 1979; Pace 1996; Calbet et al. 2001), which can, on average, account for 35% of the carbon represented by heterotrophic microorganisms in Lake Michigan in spring (Fahnenstiel et al. 1998). The preferential feeding by copepods on microzooplankton seen in this and other studies indicates that copepod grazing on protozoans can provide a mechanism for transport of the carbon found in smaller size fractions to higher trophic levels (e.g., Sherr et al. 1986; Hartmann et al. 1993). The mean clearance rates that we observed of 22 mL·copepod^{-1}·day^{-1} for copepods feeding on microzooplankton in natural seston are similar to those seen in other lakes under warmer conditions (e.g., Burns and Gilbert 1993; Jack and Gilbert 1997). Clearance rates of microzooplankton by the calanoid *Eudiaptomus gracilis* in late spring (temperature range 8.5–15.1 °C) can be 5.6 times higher than clearance rates of Chl *a* (Adrian and Schneider-Olt 1999). In the present study, the average ratio of clearance rates of copepods feeding on microzooplankton versus phytoplankton for any size fraction of Chl *a* was approximately 6.5, and our data show that, even when similarly sized phytoplankton are abundant, microzooplankton are preferred prey.

The majority of the large ciliates (e.g., oligotrichs and tintinnids) consumed by *L. sicilis* in these experiments were herbivorous or omnivorous, and therefore may have repackaged primary production, as well as bacterial production. Therefore, the picoplankton–protozoan–copepod coupling in Lake Michigan postulated by Carrick et al. (1991) is supported by the present study, where between 22% and 74% of the carbon ingested by copepods is microzooplankton carbon. Terrestrial inputs of dissolved organic carbon and phosphorus (e.g., from riverine and groundwater inputs and from resuspension events) may stimulate bacterial production (Cotner et al. 2000; Biddanda and Cotner 2002) and alter phytoplankton and microzooplankton species composition, particularly in shallow (<20 m) regions of the lake (Fahnenstiel et al. 2001; Julius and Goad 2001). Our experiments support the possibility that an increase in bacterial productivity and cell size during resuspension events, as seen during the EEGLE study (Biddanda and Cotner 2002) provides a potential carbon source to planktonic grazers through the microzooplankton–copepod link.

Preferential feeding on microzooplankton may reflect nutritional requirements of the copepod, because feeding on ciliates and heterotrophic dinoflagellates when nutritious phytoplankton are not available has been shown to increase copepod secondary production (Kleppel 1993; Kiørboe and Nielsen 1994; Ederington et al. 1995). Assuming a body weight of 6.6 μg C·copepod^{-1} (H.A. Vanderploeg, unpublished data), a specific growth rate of 0.03·day^{-1}, and an estimated growth efficiency of 0.3 for freshwater copepods at low temperatures (Hansen and Christoffersen 1995), *L. sicilis* females require 0.66 μg C·copepod^{-1}·day^{-1}, or 10% of body carbon·day^{-1} to support growth. Because calanoids reach a terminal molt at the adult stage, the majority of female growth is expressed as reproduction. In this study, copepods obtained 0.6%–2.2% and 0.6%–4.2% of body carbon·day^{-1} from microzooplankton and phytoplankton, respectively, with the carbon intake from both sources combined ranging from 2.0%–6.3% of body carbon·day^{-1} (mean, 4.1%). These estimates are based on an estimated C : Chl *a* ratio of 20, calculated empirically from cell counts and cell volumes. If we were to use a C : Chl *a* ratio of 50 as a conservative estimate, mean total carbon intake would increase to 0.5 μg C·copepod^{-1}·day^{-1}, or 7.1% of body C·day^{-1}. In only one experiment (12 March 2000 at station NB20) was intake of phytoplankton biomass sufficient to support estimated copepod reproduction. Therefore we conclude that, in spite of an abundance of phytoplankton carbon, copepods were potentially food-limited during these experiments, microzooplankton carbon contributed significantly to carbon demand, and an additional supplement from lipid reserves (Vanderploeg et al. 1992*b*) would have been required to support reproduction.

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In conclusion, our results show that microzooplankton are a significant resource for copepods in Lake Michigan before and during the spring bloom in Lake Michigan. The capacity to utilize both microzooplankton and phytoplankton as important carbon sources allows *L. sicilis* to take advantage of episodic increases in heterotrophic production at a time in the year when competitors and predators are in low abundance and phytoplankton assemblages may be limited by light, temperature, or nutrients. Recent modeling efforts support this conclusion (Chen et al. 2002; Ji et al. 2002) and indicate that our findings may be applicable to other systems in which periodic physical mixing events replenish depleted nutrients, stimulate heterotrophic productivity, and lengthen the food chain to include copepods and other mesozooplankton, which in turn become links to upper trophic levels.

Acknowledgements

We thank Debra Hersha for assistance with feeding experiments and with microscopic enumeration of picoplankton. The crew and engineers of the R/V *Lake Guardian* and R/V *Laurentian* facilitated plankton collections and made it possible to conduct experiments under challenging physical conditions. We also thank Steve Ruberg, Tom Johengen, Megan Agy, and Joann Cavaletto for their help with data collection and analyses, and Dr. G.-A. Paffenhöfer for helpful comments on the manuscript.

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Modelling the invasion risk of diapausing organisms transported in ballast sediments

Marjorie J. Wonham, Sarah A. Bailey, Hugh J. MacIsaac, and Mark A. Lewis

Abstract: Biological invasions via ship ballast are a major driver of biodiversity change in aquatic systems. In the Laurentian Great Lakes basin, unique shipping operations pose a particular invasion risk. The majority (>90%) of incoming vessels are not fully loaded with ballast water but carry ballast sediments and residual water. Ballast sediments contain viable diapause organisms that could hatch and be released during routine ballast operations. To assess invasion risk and control of this pathway, we constructed and parameterized a dynamic hatching model for three representative groups of freshwater organisms that undergo diapause: rotifers, cladocerans, and copepods. Numerical model simulations show that ballast sediment is a plausible invasion pathway for all three groups. The model also illustrates that postuptake ballast treatment to remove or kill potential invaders should be applied as late as possible prior to deballasting to minimize the number of live organisms released. Simulations show that the ability of taxa to reproduce parthenogenetically inside a ballast tank greatly influences quantitative predictions of both risk and control.

Résumé : Les invasions biologiques par l'intermédiaire des eaux de ballastage des navires sont des causes majeures de changements de biodiversité dans les systèmes aquatiques. Dans le bassin hydrographique des Grands Lacs laurentiens, des opérations de transport maritime particulières posent une risque spécial d'invasion. La majorité (>90 %) des navires qui y pénètrent ne portent pas une pleine charge d'eau de ballastage, mais contiennent des sédiments et de l'eau résiduelle dans leurs ballasts. Les sédiments dans les ballasts contiennent des organismes viables en diapause qui peuvent éclore et être libérés durant les opérations de ballastage de routine. Afin d'évaluer le risque d'invasion et le contrôle de cette voie d'introduction, nous avons construit un modèle dynamique de l'éclosion pour trois groupes d'organismes d'eau douce qui subissent la diapause, les rotifères, les cladocères et les copépodes, et nous en avons précisé les paramètres. Des simulations du modèle numérique montrent que les sédiments des ballasts sont une voie plausible d'invasion pour l'ensemble des trois groupes. Le modèle démontre aussi que le traitement des eaux de ballastage après le remplissage afin d'éliminer ou de tuer les organismes envahisseurs possibles devrait se faire le plus tard possible avant la vidange des ballasts, afin de réduire au minimum le nombre d'organismes vivants libérés. Les simulations montrent que la capacité des taxons de se reproduire par parthénogenèse dans les ballasts influence fortement les prédictions numériques à la fois du risque et du contrôle.

[Traduit par la Rédaction]

Introduction

Biological invasions are one of the major agents of global biodiversity change in aquatic systems (e.g., Sala et al. 2000; MacIsaac et al. 2001). In the Laurentian Great Lakes basin, current invasion rates exceed background by several orders of magnitude, and published records document at least 170 established nonindigenous species (Ricciardi 2001; Hebert and Cristescu 2002; Holeck et al. 2004). Because anthropogenic invasion pathways remain active, new species are predicted to invade the Great Lakes successfully in the future (Table 1) (Vanderploeg et al. 2002; Grigorovich et al. 2003; Rixon et al. 2005).

Ballast water is widely recognized as the major introduction pathway in many aquatic systems (Carlton 1998; MacIsaac et al. 2002; Grigorovich et al. 2003). In the Great Lakes, however, ship surveys indicate that fewer than 10% of commercial vessels entering the lakes are registered in

Received 14 October 2004. Accepted 22 April 2005. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on 24 September 2005.
J18355

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 doi: 10.1139/F05-146 © 2005 NRC Canada

Table 1. Nonindigenous species with diapause stages established (E) in the Laurentian Great Lakes, nonindigenous species predicted (P) to invade from the Ponto-Caspian region, and viable diapausing taxa collected (C) in ballast sediments arriving in the Great Lakes (Ricciardi 2001; Bailey et al. 2003; Grigorovich et al. 2003) and the origin for established and predicted invaders.

Group	Species	E	P	C	Origin
Rotifers	*Asplanchna girodi*			×	
	Brachionus angularis			×	
	Brachionus budapestinensis			×	
	Brachionus calyciflorus			×	
	Brachionus diversicornis			×	
	Filina sp.			×	
	Ploesoma sp.			×	
Cladocerans	*Bythotrephes longimanus*	×			Eurasia
	Bosmina coregoni	×			Eurasia
	Bosmina maritima	×			Eurasia
	Cercopagis pengoi	×			Black Sea
	Daphnia lumholtzi	×			Africa, Asia
	Bosmina obtusirostris		×		Ponto-Caspian
	Podonadne trigona ovum		×		Ponto-Caspian
	Cornigerius maeoticus maeoticus		×		Ponto-Caspian
	Daphnia cristata		×		Ponto-Caspian
	Bosmina liederi			×	
	Bosmina sp.			×	
	Ceriodaphnia pulchella			×	
	Daphnia ambigua			×	
	Daphnia longiremis			×	
	Daphnia magna			×	
	Daphnia sp.			×	
	Diaphanosoma brachyurum			×	
	Diaphanosoma sp.			×	
	Moina affinis			×	
	Moina micrura			×	
Copepods					
Calanoids	*Eurytemora affinis*	×			Cosmopolitan
	Skistodiaptomus pallidus	×			North America
	Calanipedia aquaedulcis		×		Ponto-Caspian
	Heterocope appendiculata		×		Ponto-Caspian
	Heterocope caspia		×		Ponto-Caspian
	Unidentified calanoids			×	
Harpacticoids	*Heteropsyllus cf. nunni*	×			Ponto-Caspian
	Nitocra hibernica	×			Ponto-Caspian
	Nitocra incerta	×			Ponto-Caspian
	Onychocamptus mohammed	×			Ponto-Caspian
	Schizopera borutzkyi	×			Ponto-Caspian
	Ectinosoma abrau		×		Ponto-Caspian
	Paraleptastacus spinicaudata triseta		×		Ponto-Caspian
Cyclopoids	*Cyclops strenuus*	×			Eurasia
	Megacyclops viridis	×			Europe
	Cyclops kolensis		×		Ponto-Caspian

ballast (Colautti et al. 2003). The remaining >90% are registered as NOBOB (no ballast on board), but nonetheless carry, on average, some 10–15 metric tons (t) of sediment and 40–50 t of residual, unpumpable ballast water (Bailey et al. 2003; Colautti et al. 2003). Both the sediment and residual water may contain live organisms and viable resting stages (e.g., Bailey et al. 2003, 2005*a*; Colautti et al. 2003). By loading and discharging ballast water within the Great Lakes, NOBOB vessels provide the opportunity for nonindigenous species to be released from these ballast residuals (MacIsaac et al. 2002; Colautti et al. 2003; Bailey et al. 2005*b*). The relative risk of invasion via ballast water, ballast sediment, and residual ballast water remains unknown. Here, we focus on characterizing the invasion risk posed by the diapausing freshwater organisms in ballast sediment, up to the point of release into the new environment.

Sediment accumulates in ballast tanks during the years between dry dock cleanings and contains a variable density

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and diversity of live organisms as well as the viable diapause stages of diatoms, dinoflagellates, ciliates, flagellates, and invertebrates (e.g., Smith et al. 1999; Bailey et al. 2003, 2005*a*). Diapausing eggs and other resting stages provide a temporal refuge from adverse environmental conditions, particularly between temperate growing seasons. They may also predispose species to successful transport and possible invasion via human activities.

In general, diapausing eggs undergo an obligate resting, or refractory, period of weeks to months before becoming receptive to hatching cues. They can then hatch out in response to a variety of factors including light, temperature, salinity, and oxygen (e.g., Hairston et al. 1995; Cáceres 1997; Gray et al. 2005). If the ballast tanks of NOBOB vessels remained sealed through the Great Lakes, diapausing organisms would pose no invasion risk. However, the addition of water while operating on the Great Lakes can alter conditions in the tanks and stimulate hatching (Bailey et al. 2005*b*), allowing organisms to be released.

To assess the invasion risk associated with diapausing stages of freshwater organisms in ballast sediments, we construct a dynamic model of hatching within ballast tanks of NOBOB vessels entering the Great Lakes. Our goal is to explore the simplest-case scenarios of single-species population growth and control for ballast sediment organisms. We parameterize our model for several common diapausing species found in ballast sediments or introduced into the Great Lakes. We then identify the optimal timing of ballast treatment to minimize the release of live organisms.

Model development

Invasion pathway

Most NOBOB ships in the Great Lakes discharge cargo and load ballast water at all but the last port of call, where the majority then load cargo and discharge the accumulated water. Most (84%) first ports of call are on Lakes Ontario and Erie, and most (74%) ballast discharges occur in Lake Superior (for vessels entering the Great Lakes from 1994 to 2000; Colautti et al. 2003). The average ship transit time from water intake in western Lake Ontario to discharge in Lake Superior is 7–14 days, including stops at other ports (Bailey et al. 2003). Laboratory and ballast tank studies show that resting stages of freshwater taxa may hatch in as few as 2 days following freshwater immersion, which would allow eggs to hatch in Great Lakes ballast well before final discharge (Bailey et al. 2003, 2005*b*).

Physical conditions in ballast tanks are variable, and although heavy mortality is typically observed, the occasional presence of gravid females, loose eggs, and early larval stages suggests that successful reproduction may occur in a variety of taxa (e.g., Smith et al. 1999; Gollasch et al. 2000; Wonham et al. 2001). We therefore consider two hypothetical ballast tank scenarios: (*i*) conditions that allow sediment organisms to hatch and develop from diapausing eggs but not to reproduce and (*ii*) conditions that allow hatching, development, and reproduction.

The majority of diapausing eggs in incoming ballast sediments to the Great Lakes are rotifers and cladocerans (Table 1) (Bailey et al. 2003, 2005*a*). Copepod eggs are found less frequently in sediments, but live individuals are nearly ubiquitous in ballast water samples (e.g., Smith et al. 1999; Gollasch et al. 2000; Wonham et al. 2001). Within these three groups, we summarized records of nonindigenous species established in or predicted to invade the Great Lakes and viable taxa collected as diapausing eggs from ballast sediments entering the lakes (Table 1). Rotifer species were commonly collected from ballast sediments (seven taxa), but no established rotifer invaders were reported from this region. Cladoceran species were also commonly collected (11 taxa), although typically at low abundance, and were commonly reported or predicted to invade (nine species). Copepods were also commonly reported and predicted to invade (15 species); although diapausing eggs have been collected frequently from ballast sediments,

Fig. 1. (*a*) Simplified life cycle of parthenogenetic organisms hatching from diapausing eggs in ballast sediments, including stages and rate parameters. In model 1 (solid boxes and lines), diapausing eggs hatch to larval or juvenile stages following an appropriate environmental cue and then mature to adults. Adult females typically produce parthenogenetic eggs; sexual diapausing eggs may be produced later, but as this is unlikely to occur within the relatively short time frame of ballast transport in the Great Lakes, it is not included in the model. For copepods, we assume that sexual egg production can be approximated in the confines of a ballast tank by parthenogenesis. In model 2, a developing egg compartment is added (broken boxes and lines). (*b*) Both convex (solid line) and sigmoid (broken line) cumulative hatching profiles are observed in the laboratory for different species and can be generated by models 1 and 2, respectively.

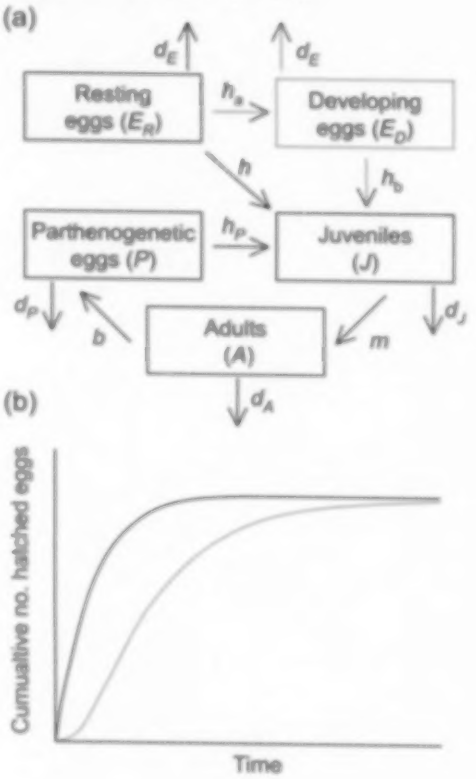

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Table 2. Diapausing egg hatching parameters estimated from the literature for species hatching from ballast sediments.

Species	t_{min}	$\bar{t}$	h	n	Reference(s)
Asplanchna girodi	1	1.30	1.460	86	*a*
Brachionus budapestinensis	1	1.80	0.809	61	*a*
Brachionus calyciflorus	0	2.07	0.658	56	*a*
Bosmina liederi	2	2.16	0.623	51	*a*
Bythotrephes longimanus	24	46.66	0.022	29	*b*
Daphnia longiremis	2	5.56	0.198	25	*a*
Eurytemora affinis	0.75	1.42	1.216	30	*c*

Note: t_{min}, first observed hatching day minus one, following the cue; $\bar{t}$, mean number of days to hatching following t_{min}; h, maximum-likelihood estimated of daily per capita hatching rate; n, total number of hatched eggs. References: (*a*) Bailey et al. (2003) and S. Bailey, unpublished data; (*b*) Yurista (1992); and (*c*) Ban and Minoda (1992).

Table 3. Literature data for species collected from ballast sediments (or for related species if data unavailable).

Species	Juvenile duration (days)	Adult life span (days)	Parthenogenetic eggs (days)	Fecundity (no. of eggs)	Reference(s)
Asplanchna girodi	—	4.8–8.7	—	15.6–21.7·female^{-1}	*a*
Brachionus calyciflorus	0.8–1.0	1–9	0.7–1.2	3.6–34.1·female^{-1}	*b*
Bosmina longisrostris	1–8	10–30	2.1–5.4	3·female^{-1}·day^{-1}	*c*
Bythotrephes longimanus	14–23	6.4–13.0	11–14	—	*d*
Daphnia spp.	2–17	8–70	2–4	0.1–4.4·female^{-1}·day^{-1}	*e*
Eurytemora affinis	11.4–37	5–73	1.0–2.2[a]	2.1–34.2·female^{-1}·day^{-1}	*f*

Note: Daily per capita rate parameters were taken as follows: juvenile maturation rate, m, as 1 over the juvenile duration; adult mortality rate, d_A, as 1 over the adult life span; and parthenogenetic hatching rate, h_p, as 1 over the egg development time. Parthenogenetic fecundity is given as eggs per female per day or total number of eggs per female, as indicated; daily parthenogenetic fecundity ($1/b$) was estimated where necessary by dividing total fecundity by mean adult life span. Parameter values used in the simulations are given in the figure captions. References: (*a*) Sarma et al. (2003); (*b*) Pourriot et al. (1982), Herzig (1983), Rothhaupt (1990), Fernández-Casalderrey et al. (1993), Hettiarachchi et al. (1995), Mnatsakanova and Polishchuk (1996), Wang and Li (1997), and Xi and Huang (2001); (*c*) Kwik and Carter (1975), Lynch (1980), Hanazato and Yasuno (1985), Urabe (1991), and McCauley et al. (1996); (*d*) Sprules et al. (1990), Yurista (1992), Lehman and Branstrator (1995), Yurista and Schulz (1995), and Lehman et al. (1997); (*e*) Lynch (1980), Gurney et al. (1990), McCauley et al. (1990*a*, 1990*b*), Teschner (1995), Arbaciauskas and Gasiûnaitë (1996), and McCauley et al. (1996); and (*f*) Heinle and Flemer (1975), Allan and Daniels (1982), Gaedke (1990), Ban (1992), Hirche (1992), Nagaraj (1992), Koski et al. (1999), Lee and Petersen (2002), Reinikainen et al. (2002), and Hairston et al. (1995).

[a]For the copepod *E. affinis*, we use sexual egg development time.

species identifications are few (Table 1). We chose representative species from each group to model their relative invasion risk from diapausing eggs in ballast sediments.

Model structure

We represent the general rotifer, cladoceran, and copepod life cycle as a simplified cycle of four stages, which can be modelled with a system of four ordinary differential equations (Fig. 1*a*; eq. 1). We consider an initial population of viable diapausing eggs (E_R) that hatch in response to an environmental cue into a larval or juvenile stage (J), mature to adults (A), and produce parthenogenetic eggs (P) (Fig. 1*a*):

$$(1a)\quad \underbrace{\frac{dE_R}{dt}}_{\text{resting eggs}} = -\underbrace{\frac{hE_R}{2}}_{\text{hatching}} - \underbrace{d_E E_R}_{\text{mortality}}, \qquad h = \begin{cases} 0, & t < t_{min} \\ h, & t \geq t_{min} \end{cases}$$

$$(1b)\quad \underbrace{\frac{dJ}{dt}}_{\text{juveniles}} = \underbrace{\frac{hE_R}{2}}_{\text{hatching from resting eggs}} + \underbrace{h_P P}_{\text{hatching from parthenogenetic eggs}} - \underbrace{mJ}_{\text{maturation of juveniles}} - \underbrace{d_J J}_{\text{mortality}}$$

$$(1c)\quad \underbrace{\frac{dA}{dt}}_{\text{adults}} = \underbrace{mJ}_{\text{maturation of juveniles}} - \underbrace{d_A A}_{\text{mortality}}$$

$$(1d)\quad \underbrace{\frac{dP}{dt}}_{\text{parthenogenetic eggs}} = \underbrace{bA}_{\text{production by adult females}} - \underbrace{h_P P}_{\text{hatching}} - \underbrace{d_P P}_{\text{mortality}}$$

Parameters are further defined in Tables 2 and 3. For copepods, which do not reproduce parthenogenetically, we make the simplifying assumption here that egg fertilization in the confines of a ballast tank is not limited by sperm availability, so sexual reproduction can be approximated by parthenogenesis.

We treat the hatching of diapausing eggs as a series of independent, one-step events that can be modelled as a Poisson process, which generates an exponential distribution of egg hatching times. This distribution is modelled with the constant hatching rate parameter h (eqs. 1*a* and 1*b*). The resulting convex profile of cumulative eggs hatched over time is consistent with observed laboratory data for certain species (e.g., Bailey et al. 2003) and can be visualized by setting all other parameters to zero and solving for the total

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number of hatched organisms, $J(t) = E_0(0)(1 - e^{-ht})$ (Fig. 1*b*).

Other species show a sigmoid cumulative hatching profile (Ban and Minoda 1992; Yurista 1992, 1997). To better account for this shape, we developed a second model that considers egg development as a two-stage process (Fig. 1; Appendix A). Since this more complex model was not supported significantly better by the data (Appendix A), we use the simpler model throughout.

The life cycle model assumes that the species' life stages are adequately represented by these equations, that sexual diapausing egg production is insignificant in the short time frame of interest, and that all parameter values remain constant over time (cf. Arbaciauskas and Gasiûnaitë 1996). We note that by using experimental laboratory data to estimate parameter values, we may be overestimating survival and development rates (Bailey et al. 2005*a*). Nonetheless, our results remain qualitatively similar over a range of parameter values. We use this simple deterministic model to simulate a 14-day ballast water voyage through the Great Lakes in which the environmental hatching cue occurs on the first day.

Parameter estimation

We used laboratory and literature data to obtain parameter estimates for representative species from Table 1 (Tables 2 and 3; Fig. 2). For diapausing eggs, we estimated the mean time to hatching based on literature and laboratory data. From each study, we assumed that only those eggs hatching within the study period were viable (i.e., that no more would have hatched had the experiment been longer). For studies that reported multiple laboratory trials, we preferentially chose those conducted at 0‰ salinity, 10–20 °C, and 24 h dark regimes. We pooled data from multiple replicates and studies to obtain a single hatching rate estimate for each species using maximum likelihood. There were insufficient data available to estimate parameter distributions. For each species, the initial delay before hatching, t_{min}, was taken as the day prior to the first reported hatching. The hatching time, t_i, for each egg i (up to n, the total number that hatched in the laboratory trial) was calculated as the observed hatching day less t_{min}. The mean observed hatching time is denoted $\bar{t} = \sum_{i=1}^{n} t_i/n$.

Since hatching is a continuous process through time, we represent it with an ordinary differential equation model (eq. 1). However, the laboratory data used to estimate hatching rates are typically recorded at discrete daily time points, where each observation represents the number of eggs hatched in the previous day. To estimate hatching rates, we determined the continuous probability density function (pdf) for hatching times, integrated this to obtain the probability of an egg hatching in a given 1-day period, and calculated the likelihood function for the hatching rate. The time to hatching, T, has pdf he^{-ht}. The probability of an egg hatching during a 1-day period from $j - 1$ to j is thus

$$(2) \qquad \Pr\{j-1 < T < j\} = \int_{j-1}^{j} he^{-ht}dt = e^{-ht}(e^{h} - 1)$$

Given observed hatching times t_i, the negative log-likelihood function for the hatching rate h is

$$(3) \qquad -l(h|t_1,\ldots,t_n) = -\ln\left[\prod_{i=1}^{n} e^{-ht_i}(e^{h} - 1)\right] = -n\ln(e^{h} - 1) + ht\bar{t}$$

By minimizing this function, we obtained hatching rate estimates (Table 2; Fig. 2; Appendix A).

The remaining life cycle parameter estimates were obtained from the literature (Table 3). When data were not available for a particular species, values from closely related species were used as follows: *Asplanchna girodi* juvenile and parthenogenetic egg durations were based on those of *Brachionus calyciflorus*; all *Brachionus* spp. life cycle parameters were based on those of *Brachionus calyciflorus*; *Bosmina liederi* life cycle parameters were based on those of *Bosmina longirostris*; and *Daphnia longiremis* life cycle parameters were based on those of *Daphnia galeata* and *Daphnia pulex* (Tables 2 and 3).

Estimates of mortality rates at egg and juvenile stages were generally not available, so for most simulations, d_E, d_P, and d_J were set to zero. The effects of additional mortality were explored for *Eurytemora affinis* by adding juvenile mortality, $d_J = 0.2 \cdot \text{day}^{-1}$, and reducing egg viability to 70% (as per sources in Table 3; Heinle and Flemer 1975).

Model analysis

We first simulated ballast tank conditions in which individuals could hatch and develop but not reproduce. For the six species shown, the brief period from the hatching cue to the first juveniles corresponded to t_{min} (Fig. 3). Rotifer abundance increased and then declined by the end of the 14-day simulation as the supply of diapausing eggs was depleted (Figs. 3*a*–3*c*). The two cladocerans *Bosmina liederi* and *D. longiremis*, which hatched and developed more slowly, showed a similar but slower trend (Figs. 3*d* and 3*e*). For the copepod *E. affinis*, which hatched relatively quickly but had a longer development period to reproductive maturity, only the juveniles began to decline within the 14-day simulation (Fig. 3*f*).

When we allowed parthenogenetic reproduction to occur, all six species produced new generations within the model time frame (Fig. 4). Again, the rotifer populations increased the fastest, followed by the cladocerans and the copepod (Figs. 4*a*–4*f*). Adding juvenile and parthenogenetic egg mortality for *E. affinis* reduced the final hatched populations fivefold in both scenarios (Figs. 3*g* and 4*g*). The cladoceran *Bythotrephes longimanus*, which had the longest t_{min} and the slowest hatching rate of the species considered, would be unable to hatch from diapausing eggs within 14 days using these parameter values (Tables 2 and 3). Even when hatching and development rates were reduced by an order of magnitude, all of the taxa illustrated in Figs. 3 and 4 were capable of hatching within the model time frame (results not shown).

A variety of physical and chemical ballast water treatments have been proposed to reduce invasion risk (e.g., Kuzirian et al. 2001; Tamburri et al. 2002; Waite et al. 2003). We asked how the timing of treatment influenced its effectiveness in minimizing the final abundance of live organisms. For simplicity, we assume that treatment kills a

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Fig. 2. Observed (points) and predicted (solid lines, model 1; broken lines, model 2) number of diapause eggs hatching on each day following t_{min} for (*a*) *Asplanchna girodi*, (*b*) *Brachionus budapenstensis*, (*c*) *Brachionus calyciflorus*, (*d*) *Bosmina liederi*, (*e*) *Bythotrephes longimanus*, (*f*) *Daphnia longiremis*, and (g) *Eurytemora affinis*. The original authors collected data daily for Figs. 2*a*–2*d* and 2*f*; we assumed the same for Fig. 2*e*. Model 1 fit the data significantly better than model 2 (Appendix A) and is used for all simulations.

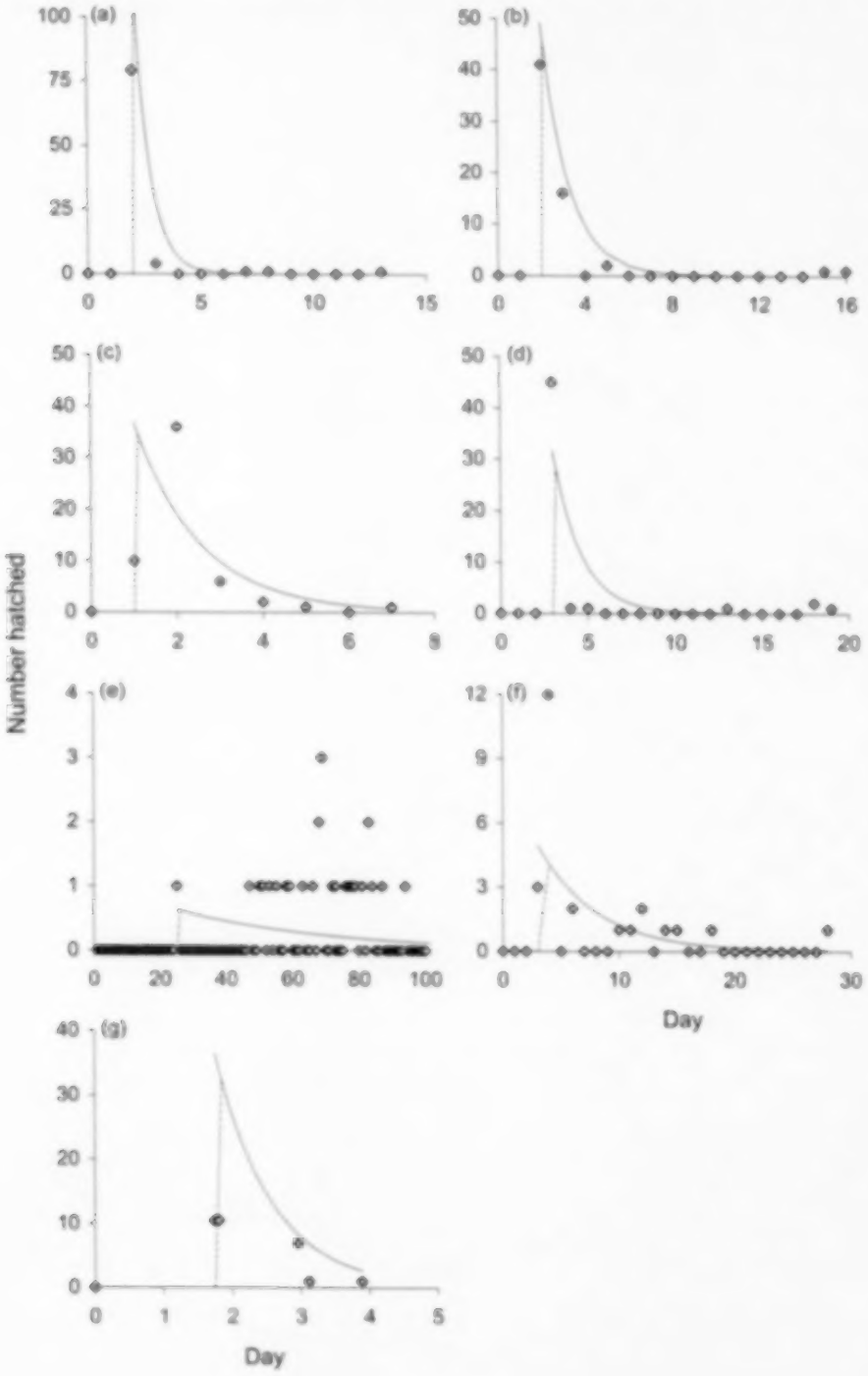

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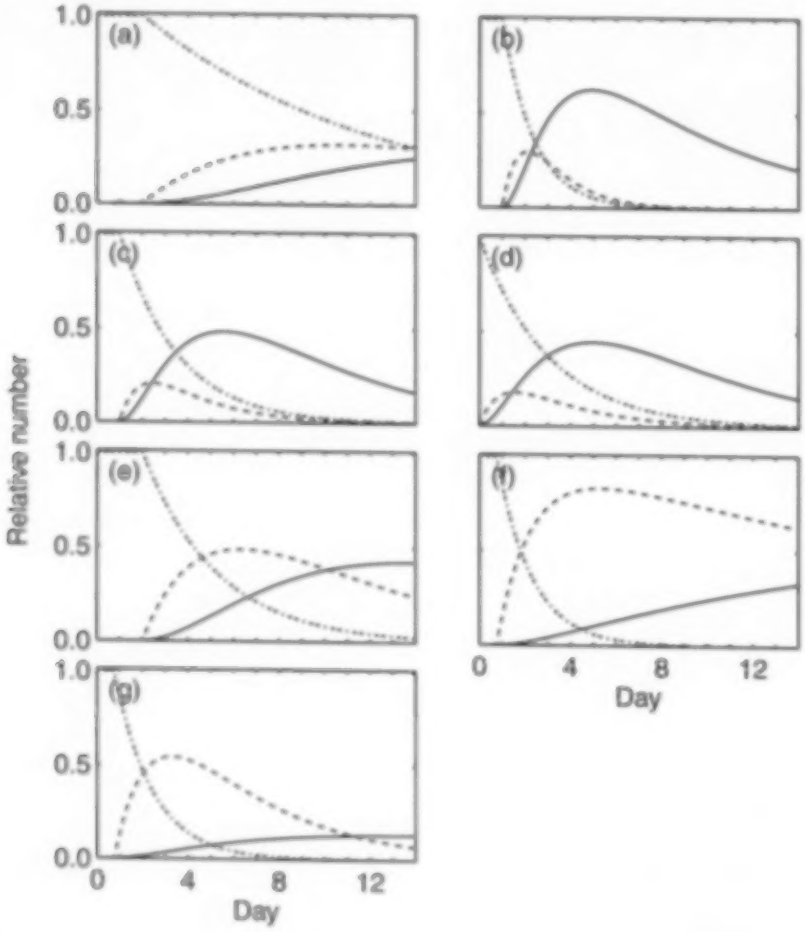
Fig. 3. Simulated population dynamics of six representative species hatching from ballast sediments under tank conditions in which they were allowed to hatch and develop but not reproduce. The numbers of viable diapausing eggs (dotted line), juveniles (dashed line), and adults (solid line) are scaled to the initial number of viable diapausing eggs on the *y* axis. Days following hatching cue on the *x* axis. Daily per capita rates and other parameters are as follows (minimum time to hatching from diapause egg, t_{min}; diapause hatching rate, h; juvenile maturation rate, m; adult mortality rate, d_A): (*a*) *Asplanchna girodi* (1, 1.460, 1.11, 0.15); (*b*) *Brachionus budapestinensis* (0, 0.658, 1.1, 0.20); (*c*) *Brachionus calyciflorus* (1, 0.809, 1.1, 0.20); (*d*) *Bosmina liederi* (2, 0.623, 0.28, 0.05); (*e*) *Daphnia longiremis* (2, 0.198, 0.13, 0.10); (*f*) *Eurytemora affinis* (0.75, 1.216, 0.04, 0.02); and (*g*) *Eurytemora affinis* with added juvenile mortality, d_J (0.75, 1.216, 0.04, 0.02, d_J = 0.2). In all cases, mortality rates of diapausing eggs are zero, and in all cases except (*g*), the juvenile mortality rate is zero.



fixed proportion of juveniles, adults, and parthenogenetic eggs but does not affect resting or developing diapausing eggs. We also assume that the treatment is effective only on the day of application and that hatching and development rates are the same before and after treatment.

Clearly, any treatment during ballast uptake will reduce the initial ballast population and therefore the subsequent propagule pressure. Once ballast is in the tank, however, treatment of a population later rather than earlier will always result in a lower abundance of live organisms. This is due to the cooperative (positive feedback) nature of this system in which eggs become juveniles, which in turn become adults, which produce more juveniles (Smith and Waltman 1995). Example results for two species illustrate how successful reproduction can influence the relative abundance of different taxa following treatment (Fig. 5). For the same initial population size, and in the absence of reproduction, final abundance was higher for the cladoceran *D. longiremis* than for the rotifer *Brachionus calyciflorus* (Fig. 5*a*). This is because the cladoceran hatches more slowly, so the pool of diapause eggs available to repopulate the tank following treatment persists longer. With reproduction, however, the abundance of the faster-developing *Brachionus calyciflorus* was higher than that of *D. longiremis* (Fig. 5*b*).

Discussion

Preventing future invasions requires understanding and interrupting the invasion process. The success of an invasion

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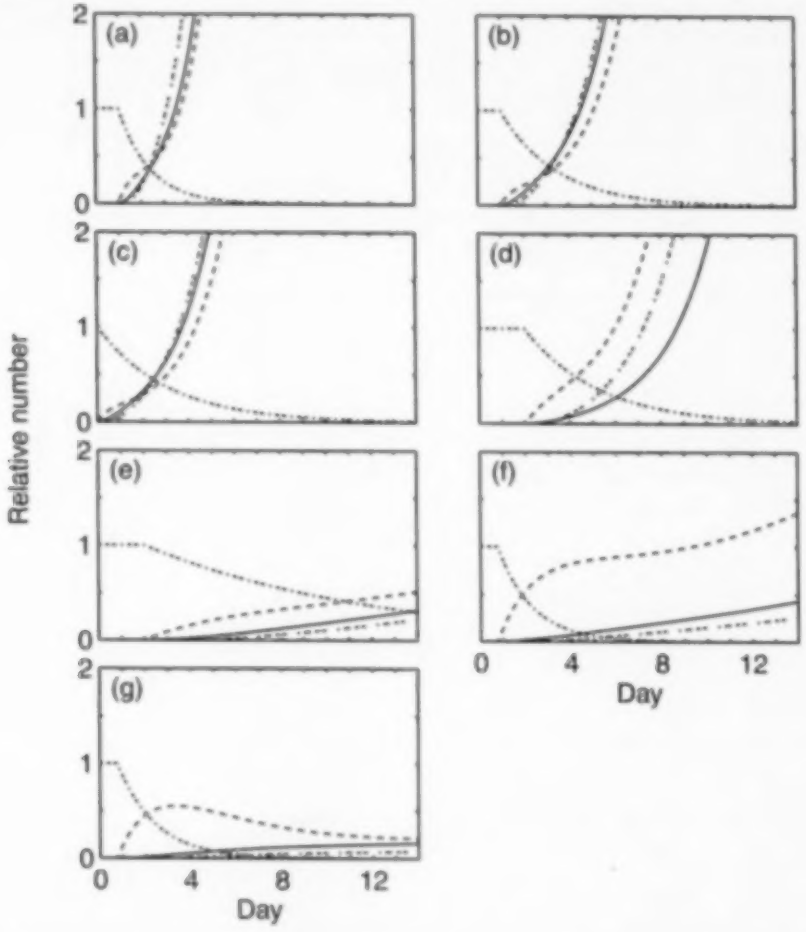
Fig. 4. Simulated population dynamics of six representative species under tank conditions in which parthenogenetic reproduction occurs. The numbers of viable diapausing eggs (dotted line), parthenogenetic eggs (dot-dashed line), juveniles (dashed line), and adults (solid line) are scaled to the initial number of viable diapausing eggs on the *y* axis. Days following hatching cue on the *x* axis. Daily per capita rates and other parameters are as follows (minimum time to hatching from diapause eggs, t_{min}; diapause hatching rate, h; juvenile maturation rate, m; adult mortality rate, d_A; parthenogentic egg production rate, b; parthenogenetic egg hatching rate, h_p): (*a*) *Asplanchna girodi* (1, 1.460, 1.11, 0.15, 2.8, 1.0); (*b*) *Brachionus budapestinensis* (0, 0.658, 1.1, 0.20, 1.8, 1.0); (*c*) *Brachionus calyciflorus* (1, 0.809, 1.1, 0.20, 1.8, 1.0); (*d*) *Bosmina liederi* (2, 0.623, 0.28, 0.05, 3.0, 0.27); (*e*) *Daphnia longiremis* (2, 0.198, 0.13, 0.10, 0.33, 0.33); (*f*) *Eurytemora affinis* (0.75, 1.216, 0.04, 0.02, 0.44, 0.62); (*g*) *Eurytemora affinis* with added juvenile mortality, d_J (0.75, 1.216, 0.04, 0.02, 0.31, 0.62, $d_J = 0.2$). In all cases, mortality rates of diapausing eggs are zero, and in all cases except (*g*), the juvenile mortality rate is zero.



depends on the invader's propagule pressure, environmental match, and biotic interactions (e.g., Williamson 1996; Ruiz et al. 2000; Rouget and Richardson 2003). With respect to propagule pressure, theory predicts that the chances of successful invasion will increase with the number of individuals released and the frequency of release (e.g., Allee et al. 1949; Grevstad 1999; Rouget and Richardson 2003). The larger and more frequent the releases, the more likely it is that the invader will overcome problems that plague small populations, such as Allee effects, genetic bottlenecks, demographic stochasticity, and environmental stochasticity. Reducing propagule pressure should therefore reduce invasion risk (e.g., Locke et al. 1993; Drake and Lodge 2004; International Maritime Organization 2004).

The propagule pressure of potential invaders of a region depends on the nature of the invasion pathways. To reduce propagule pressure and invasion risk from ship ballast in the Great Lakes, voluntary and mandatory ballast water exchange guidelines were implemented in 1989 and 1993, respectively (Locke et al. 1993). However, the expected subsequent reduction of successful invasions has not yet been seen (Ricciardi 2001; Holeck et al. 2004). We propose several nonmutually exclusive hypotheses to explain this apparent discrepancy: (1) exchange reduces invasion risk from ballast water but (*i*) other important pathways are operating, (*ii*) ballast volume or propagule quality has increased, (*iii*) the Great Lakes have become easier to invade, or (*iv*) there will be a delay before its effects are seen because

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previously introduced species are still increasing to the threshold abundance for detection; or (2) exchange does not effectively reduce invasion risk. Here, we explored the first hypothesis, namely that NOBOB vessels entering the Great Lakes may be an additional and important invasion pathway to this region (Bailey et al. 2003; Colautti et al. 2003).

From 1999 to 2000, some 400–600 vessels, both in ballast and NOBOB, entered the Great Lakes each year (Colautti et al. 2003). The invasion risk posed by these two vessel classes depends on their relative frequency and the abundance of viable organisms released. Ships in ballast represent less than 10% of incoming vessels but carry an average of 3–8.5 × 10^3 t of (exchanged) ballast water containing on the order of 10^9 bacteria·L^{-1}, 10^6 autotrophic picoplankters·L^{-1}, and 10^3 zooplankters·L^{-1} (Locke et al. 1991, 1993; MacIsaac et al. 2002). In contrast, NOBOBs make up over 90% of vessels. These ships carry an average of 10–15 t of sediment containing an average of 1.3 × 10^6 animals·t^{-1} and 7.2 × 10^5 diapausing eggs·t^{-1}, with an additional average of 47 t of residual water containing 1.1 × 10^4 animals·t^{-1} (Bailey et al. 2003, 2005*a*; Colautti et al. 2003). The invasion risk associated with each vessel type depends, ultimately, on the number of viable individuals of each species that are released. Here, we have focused on characterizing the invasion risk associated with the sediment reservoir of diapausing ballast organisms.

Ballast sediment invasion risk

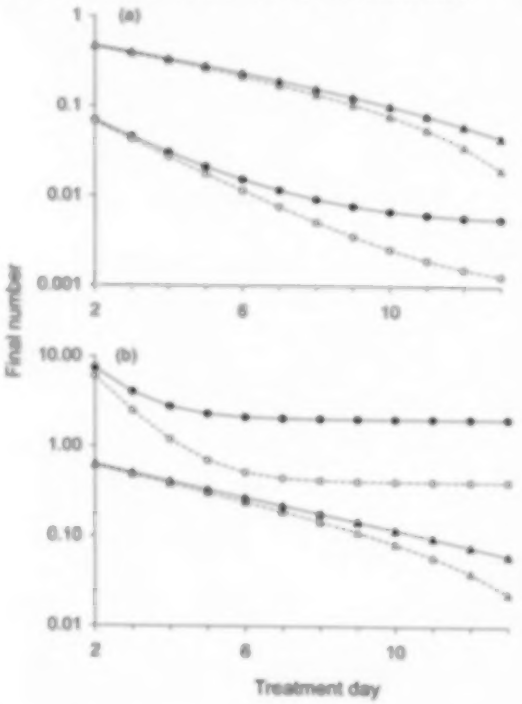
Our model clearly illustrates the potential for a range of freshwater taxa to hatch from diapause within the time frame necessary to be released in routine deballasting operations. Based on propagule pressure, our model predicts that species of all three taxonomic groups considered could readily hatch and be available for introduction from diapause populations.

Rotifers, whose diapausing eggs are abundant in ballast sediments (Bailey et al. 2003) and which grow and reproduce rapidly, would be the most likely invaders from this pathway. Although nonindigenous rotifer species have been reported from the Great Lakes and elsewhere (e.g., Josefsson and Andersson 2001; Gray et al. 2005), there has not yet been confirmation of any established populations in the Great Lakes. Since rotifers are a diverse and taxonomically challenging group, and since they may also be widely distributed by other pathways including wind (e.g., Jenkins and Underwood 1998), we suggest that past invasions may have occurred and that future genetic analysis could reveal the presence of introduced species from this group.

Cladocerans are widely collected from ballast sediments, albeit at low density, and nonindigenous cladocerans are both established in the Great Lakes and predicted to invade in the future. Their population dynamics indicate that diapausing eggs in NOBOB sediments are a possible invasion pathway. A notable exception is *Bythotrephes longimanus*, a successful invader in the lakes (e.g., Vanderploeg et al. 2002) whose lengthy development period suggests that hatched diapausing eggs from ballast sediment may have been an unlikely source.

Many nonindigenous copepods established in or predicted to invade the Great Lakes can undergo diapause, and the population dynamics of *E. affinis* indicate that ballast sediment is a possible invasion pathway. However, since copepod eggs are rare in sediments but their planktonic stages are ubiquitous in ballast water (e.g., Locke et al. 1993; Smith et al. 1999; Wonham et al. 2001), ballast water may be a more likely pathway for this group.

Fig. 5. Effects of simulated ballast water treatment on number of surviving organisms in a tank assuming (*a*) no reproduction and (*b*) parthenogenetic reproduction for the rotifer *Brachionus calyciflorus* (circles) and the cladoceran *Daphnia longiremis* (triangles). The final total number of live juveniles and adults combined is scaled to the initial number of viable diapausing eggs following a one-time 95% (solid symbols and solid line) or 99% (open symbols and broken line) mortality on each of days 2–13 of a 14-day ballast voyage. Daily per capita rates and other parameters are as follows (minimum time to hatching from diapause eggs, t_{min}; diapause hatching rate, h; juvenile maturation rate, m; adult mortality rate, d_A; parthenogenetic egg production rate, b; parthenogenetic egg hatching rate, h_p): (*a*) *Brachionus calyciflorus* (1, 0.809, 1.1, 0.20) and *D. longiremis* (2, 0.198, 0.13, 0.10); (*b*) *Brachionus calyciflorus* (1, 0.809, 1.1, 0.20, 1.8, 1.0) and *D. longiremis* (2, 0.198, 0.13, 0.10, 0.33, 0.33).



Reducing invasion risk

Complete invasion risk reduction for ballast sediments would require zero ballast discharge, but careful ballast management could nonetheless significantly reduce invasion risk. The simplest approach to risk reduction from NOBOB vessels would be to discharge water when the abundance of hatched organisms is minimal (e.g., Drake and Lodge 2004; International Maritime Organization 2004). Under the scenarios modelled here, the minimum (zero) abundance of all diapause species occurs only during the egg development pe-

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riod between the environmental cue (i.e., water uptake at first port of call) and the onset of hatching. For the invertebrate species considered here, the window is typically up to 1–3 days; for algae and microbes, it would be substantially less.

When environmental conditions in the tank do not permit parthenogenetic reproduction, a second minimal abundance of faster-developing species such as rotifers could occur towards the end of the 2-week period. In general, however, this decline should not be anticipated for the entire hatching assemblage, since the abundance of slower-growing and more starvation-resistant species such as cladocerans and copepods would remain high even after 2 weeks. Thus, the predicted optimal deballasting time for minimizing the discharge of nonreproducing diapause organisms is only during the brief time window before the eggs begin hatching. (We note, however, that if diapausing eggs are resuspended in the water during ballasting, they pose an additional invasion risk not treated here.) Considering the operational schedules of vessels operating on the Great Lakes, it would be infeasible to implement this strategy because the ballast water would, in most cases, be needed to stabilize the vessel for a longer period of time.

The physical nature of the invasion pathway can influence propagule pressure over orders of magnitude by allowing parthenogenetic reproduction (Bailey et al. 2005*b*; present study). Since we cannot assume that hatched organisms will fail to reproduce, it appears that any deballasting strategy would be most effective when combined with ballast water treatments (e.g., Kuzirian et al. 2001; Tamburri et al. 2002; Waite et al. 2003). Regardless of population dynamics, postuptake treatment should be applied as late as possible prior to deballasting (allowing time for sufficient mortality and chemical neutralization if necessary) to minimize the number of surviving organisms.

The single-species population dynamics simulated here are clearly simplifications of the multispecies interactions that would be observed in a community of organisms hatching from ballast sediments. Nonetheless, this simple modelling approach provides insights into the nature of this invasion pathway and its effective management for invasion risk reduction.

Acknowledgments

We are grateful for statistical and programming advice from C. Jerde, M. Krkosek, and P. Molnár and for data provided by I. Duggan. This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Killam Trusts (M.J.W.); an NSERC Industrial Postgraduate Scholarship in partnership with the Shipping Federation of Canada and an Ontario Graduate Scholarship (S.A.B.); NSERC, a DFO Research Chair, and the Great Lakes Protection Fund (H.J.M.); and NSERC CRO, NSF grant DEB 02-13698, and a Canada Research Chair (M.A.L.).

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Appendix A

The cumulative hatching curves reported for some diapause species appear convex (Bailey et al. 2003), but others appear sigmoid (Ban and Minoda 1992; Yurista 1992, 1997) (Fig. 1*b*). We therefore developed model 1, a single-step hatching process (eq. 1) that can generate only a concave cumulative hatching curve, and model 2, a two-step process (eq. A1) that can also generate a sigmoid hatching curve (Fig. 1):

$$\text{(A1}a\text{)}\quad \underbrace{\frac{dE_R}{dt}}_{\text{resting eggs}} = -\underbrace{\frac{h_a E_R}{2}}_{\substack{\text{initiating}\\ \text{development}}} - \underbrace{d_E E_R}_{\text{mortality}},$$

$$h_a = \begin{cases} 0, t < t_{\min} \\ h_a, t \geq t_{\min} \end{cases}$$

$$\text{(A1}b\text{)}\quad \underbrace{\frac{dE_D}{dt}}_{\substack{\text{developing}\\ \text{eggs}}} = \underbrace{\frac{h_a E_R}{2}}_{\substack{\text{initiating}\\ \text{development}\\ \text{by resting eggs}}} - \underbrace{h_b E_D}_{\text{hatching}} - \underbrace{d_E E_D}_{\text{mortality}}$$

$$\text{(A1}c\text{)}\quad \underbrace{\frac{dJ}{dt}}_{\text{juveniles}} = \underbrace{h_b E_D}_{\substack{\text{hatching from}\\ \text{developed}\\ \text{resting eggs}}} + \underbrace{h_P P}_{\substack{\text{hatching from}\\ \text{parthenogenetic}\\ \text{eggs}}} - \underbrace{mJ}_{\substack{\text{maturation}\\ \text{of juveniles}}} - \underbrace{d_J J}_{\text{mortality}}$$

The equations for adults and parthenogenetic eggs in model 2 are the same as those in model 1 (eqs. 1*c*–1*d*). Model 1 is the simplest representation of the observed transition from diapausing egg to hatched juvenile, whereas model 2 inserts a compartment for the developing embryo between the resting and hatched stages (Fig. 1*a*). Model 1 is nested within model 2 in that as $h_b \to \infty$, model 2 → model 1. In other words, as the second step of the hatching process becomes infinitely fast, model 2 reverts to model 1 (Fig. 1*b*).

Biologically, model 2 may be interpreted as, first, responding to the hatching cue by initiating development at rate h_a and, second, hatching from the egg at rate h_b. Mathematically, this formulation allows the sigmoid hatching profile that is consistent with observed laboratory data for certain species (e.g., Ban and Minoda 1992; Yurista 1992, 1997) and can be obtained by setting parameters d_E, m, and d_J to zero and solving for

$$\text{(A2)}\quad J(t) = E_R(0)\frac{h_a(e^{-h_b t} - 1) - h_b(e^{-h_a t} - 1)}{h_b - h_a}$$

For model 2, the time from diapausing egg E_R to developing egg E_D has pdf $h_a e^{-t_a h_a}$ and mean $\bar{t}_a$. The time from E_D to hatched juvenile J has pdf $h_b e^{-t_b h_b}$ and mean $\bar{t}_b$. The total waiting time from E_R to J has pdf

$$\text{(A3)}\quad f(t) = \int_0^t h_a h_b e^{-t_a h_a} e^{-(t - t_a) h_b} dt_a$$

$$= \frac{h_a h_b (e^{-h_b t} - e^{-h_a t})}{h_b - h_a}, \qquad h_b > h_a$$

with mean $\bar{t} = \bar{t}_a + \bar{t}_b$. The probability of an egg hatching in the time period $j - 1$ to j is

$$\text{(A4)}\quad \Pr\{j-1 < T < j\} = \frac{h_b}{h_b - h_a}\int_{j-1}^{j} e^{-h_b t} dt - \frac{h_a}{h_b - h_a}\int_{j-1}^{j} e^{-h_a t} dt$$

$$= \frac{h_b}{h_b - h_a} e^{-h_b j}(e^{h_b} - 1) - \frac{h_a}{h_b - h_a} e^{-h_a j}(e^{h_a} - 1)$$

The corresponding negative log-likelihood function for the two hatching rates is

$$\text{(A5)}\quad -l(h_a, h_b | t_1, \ldots, t_n) = n \ln(h_b - h_a) - \sum_{i=1}^{n} \ln[h_b e^{-t_i h_b}(e^{h_b} - 1) - h_a e^{-t_i h_b}(e^{h_b} - 1)]$$

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For all species, the maximum-likelihood estimate of h_a and h_b in model 2 was obtained as $h_b^{-1} \to 0$ and $h_a \to h$. In other words, model 1 fit the data better than model 2 in all cases (Fig. 2).

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Predicting cutthroat trout (*Oncorhynchus clarkii*) abundance in high-elevation streams: revisiting a model of translocation success

Michael K. Young, Paula M. Guenther-Gloss, and Ashley D. Ficke

Abstract: Assessing viability of stream populations of cutthroat trout (*Oncorhynchus clarkii*) and identifying streams suitable for establishing populations are priorities in the western United States, and a model was recently developed to predict translocation success (as defined by an index of population size) of two subspecies based on mean July water temperature, pool bankfull width, and deep pools counts. To determine whether the translocation model applied to streams elsewhere with more precise abundance estimates, we examined the relation between electrofishing-based estimates of cutthroat trout abundance and these habitat variables plus occupied stream length. The preferred model was (population size)$^{1/2}$ = 0.00508(stream length (m)) + 5.148 (N = 31). In contrast, a model based on data from the original translocation model included stream temperature and deep pool counts as variables. Differences in models appear to largely have a methodological rather than biological basis. Additional habitat coupled with increased habitat complexity may account for the form of the abundance – stream length relation in the electrofishing-based model. Model-derived estimates imply that many cutthroat trout populations are below thresholds associated with reduced risk of extinction. We believe that this model can reduce uncertainty about projected population sizes when selecting streams for reintroductions or evaluating unsampled streams.

Résumé : La détermination de la viabilité des populations de truites fardées (*Oncorhynchus clarkii*) des cours d'eau et l'identification des milieux d'eau courante adéquats pour l'établissement des populations constituent des priorités dans l'ouest des États-Unis; un modèle mis au point récemment permet de prédire le succès des transferts (défini par un indice de taille de population) de deux sous-espèces à partir de la température de l'eau en juillet, de la largeur des fosses à plein bord et du nombre de fosses profondes. Afin de déterminer si le modèle de transfert s'applique à des cours d'eau situés ailleurs avec de meilleures estimations de densité, nous avons examiné la relation entre les estimations d'abondance des truites fardées basées sur la pêche électrique et les variables de l'habitat ci-haut, plus la longueur du cours d'eau occupé. Le meilleur modèle est (taille de la population)$^{1/2}$ = 0,00508 (longueur du cours d'eau, en m) + 5,148 (N = 31). En revanche, un modèle basé sur les données utilisées dans le modèle original de transfert comprend comme variables la température de l'eau et le nombre de fosses profondes. Les différences entre les modèles semblent avoir une base plus méthodologique que biologique. Un nombre plus important d'habitats et une complexité plus grande des habitats peuvent peut-être expliquer la forme de la relation entre l'abondance et la longueur du cours d'eau dans le modèle basé sur la pêche électrique. Les estimations fournies par les modèles laissent croire que plusieurs des populations de truites fardées se trouvent à une densité inférieure au seuil associé à un risque réduit d'extinction. Nous croyons que ce modèle peut réduire l'incertitude reliée aux projections de tailles de populations lors du choix de cours d'eau pour fins de réintroduction ou pour l'évaluation de cours d'eau non inventoriés.

[Traduit par la Rédaction]

Introduction

Inland subspecies of cutthroat trout (*Oncorhynchus clarkii*) have substantially declined throughout most of their historical range over the last 150 years (Behnke 1992; Duff 1996). Consequently, biologists have made extensive efforts to found new populations, reestablish extirpated populations, and assess the size and probability of persistence of both (Hepworth et al. 1997; US Fish and Wildlife Service 1998; Kruse et al. 2001). Some of this work has

Received 30 July 2004. Accepted 29 June 2005. Published on the NRC Research Press Web site at http://cjm.nrc.ca on 24 September 2005.
J18240

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 doi: 10.1139/F05-149 © 2005 NRC Canada

been directed at developing models that relate cutthroat trout presence or abundance to habitat characteristics (Bozek and Rahel 1992; Kruse et al. 1997; Dunham et al. 1999). In one such model, Harig and Fausch (2002) established that the potential for small streams to harbor self-sustaining populations of greenback cutthroat trout (*Oncorhynchus clarkii stomias*) and Rio Grande cutthroat trout (*Oncorhynchus clarkii virginalis*) in Colorado and New Mexico was a function of summer water temperature, number of deep pools, and pool bankfull width. They concluded that this model could also be used to assess risk of extinction of existing populations of these and closely related subspecies in comparable environments.

The Colorado River cutthroat trout (*Oncorhynchus clarkii pleuriticus*) is among the suite of cutthroat trout subspecies in the Rocky Mountains and occupies cold, high-elevation streams in much of its remaining range (Young et al. 1996). Marked declines in its distribution relative to its historical range have led to special designation by government agencies (CRCT Task Force 2001) and to a petition for listing under the US Endangered Species Act (US Fish and Wildlife Service 2004). There have been simulations of population viability under a variety of habitat structure and reintroduction scenarios (Hilderbrand 2002, 2003) and thorough inventories of sites for introductions (CRCT Task Force 2001). For these reasons, the Harig and Fausch (2002) model (hereafter the translocation model) merits consideration by biologists working with this subspecies. In preliminary evaluations, Harig et al. (2000) used it to assess the likely success of a reintroduced population of Colorado River cutthroat trout, and Young and Guenther-Gloss (2004) demonstrated that model output was related to electrofishing-derived abundance estimates of greenback cutthroat trout populations in northern Colorado.

Initially, we assembled data on Colorado River cutthroat trout populations in Colorado and Wyoming to perform similar analyses, but these data had two crucial distinctions from those used to develop the original model. First, most of the populations that we sampled had persisted indefinitely, whereas some streams in which populations failed to become established were used to develop the translocation model. Second, trout abundance was treated as a categorical variable (i.e., levels of translocation success) in the model, whereas we chose to examine the relation of habitat to abundance analyzed as a continuous variable. This also permitted us to include similarly obtained data on greenback cutthroat trout from northern Colorado (Young and Guenther-Gloss 2004). Consequently, we broadened our goals to include examining relations between stream characteristics and cutthroat trout abundance, devising a method to estimate the abundance of cutthroat trout in unsampled streams, and providing guidance for selecting streams likely to produce populations exceeding standards for viability. Our objectives were to (*i*) develop a model relating the same habitat variables in the translocation model, plus occupied stream length, to the abundance of age-1 and older Colorado River cutthroat trout and greenback cutthroat trout sampled by electrofishing, (*ii*) attempt to validate the model by conducting a similar analysis of the data of Harig and Fausch (2002) for greenback cutthroat trout and Rio Grande cutthroat trout but limited to those streams in which translocations were at least partially successful, and (*iii*) use the preferred model for electrofishing-based estimates to predict amounts and kinds of habitat necessary to sustain populations thought to meet conservation thresholds.

Methods

Field sites

To address the first objective, we used three sets of data: electrofishing-based abundance estimates of greenback cutthroat trout in the South Platte River basin in Colorado (N = 9 streams) (Young and Guenther-Gloss 2004) and of Colorado River cutthroat trout in headwater streams in the Little Snake River basin in Wyoming (N = 12) and the upper Colorado River basin in Colorado (N = 10) (Fig. 1). To fulfill the second objective, we used the ocular counts of abundance of 21 populations of greenback cutthroat trout in the South Platte and Arkansas River basins in Colorado and of Rio Grande cutthroat trout in the Rio Grande basin in Colorado and New Mexico (Harig and Fausch 2002). All populations of Colorado River cutthroat trout in Wyoming were believed to be indigenous, whereas those in Colorado were either indigenous or had persisted for decades after stocking (Young et al. 1996). All Rio Grande cutthroat trout and most greenback cutthroat trout populations were relatively recent introductions (Harig and Fausch 2002; Young et al. 2002). Stocks represented a mixed heritage; some were believed to be genetically pure and others were introgressed with nonindigenous cutthroat trout or rainbow trout (*Oncorhynchus mykiss*).

Segments of named streams (from US Geological Survey 1 : 24 000 maps) with allopatric populations of cutthroat trout were typically treated as the sampling units. Barriers to migration (desiccated reaches, artificial barriers, or waterfalls) isolated most populations from upstream invasions of nonnative fishes. In Wyoming, two-way fish migration was possible between all tributaries and the mainstem North Fork Little Snake River (the lower boundary of which was blocked from upstream fish migration by a weir) except the upper portions of Deadman, Harrison, Ted, and Third creeks and the main stem itself, in which water diversions isolated additional populations upstream. Downstream and upstream segments in Deadman and Harrison creeks and the main stem were treated as different sampling units, whereas the short downstream segments of Ted and Third creeks (<450 m) were pooled with the main stem. In the upper North Fork Little Snake main stem and in some Colorado streams, small (<2.5 m bankfull width), short (generally <1000 m) named tributaries to the primary sampling streams were also sampled and pooled with the primary streams for our analyses.

There was broad overlap in the ranges of the physical characteristics of these streams (Table 1) and in climate, hydrology, and trout population characteristics. Most were high-elevation, perennial streams with snowmelt-dominated flows and gravel–cobble–rubble channels. Riparian overstory vegetation usually consisted of coniferous forests of Engelmann spruce (*Picea engelmannii*), subalpine fir (*Abies lasiocarpa*), and lodgepole pine (*Pinus contorta*) interspersed with sites dominated by quaking aspen (*Populus tremuloides*), deciduous shrubs, and meadows. Because most streams contained barriers, cutthroat trout probably exhibited resident life histo-

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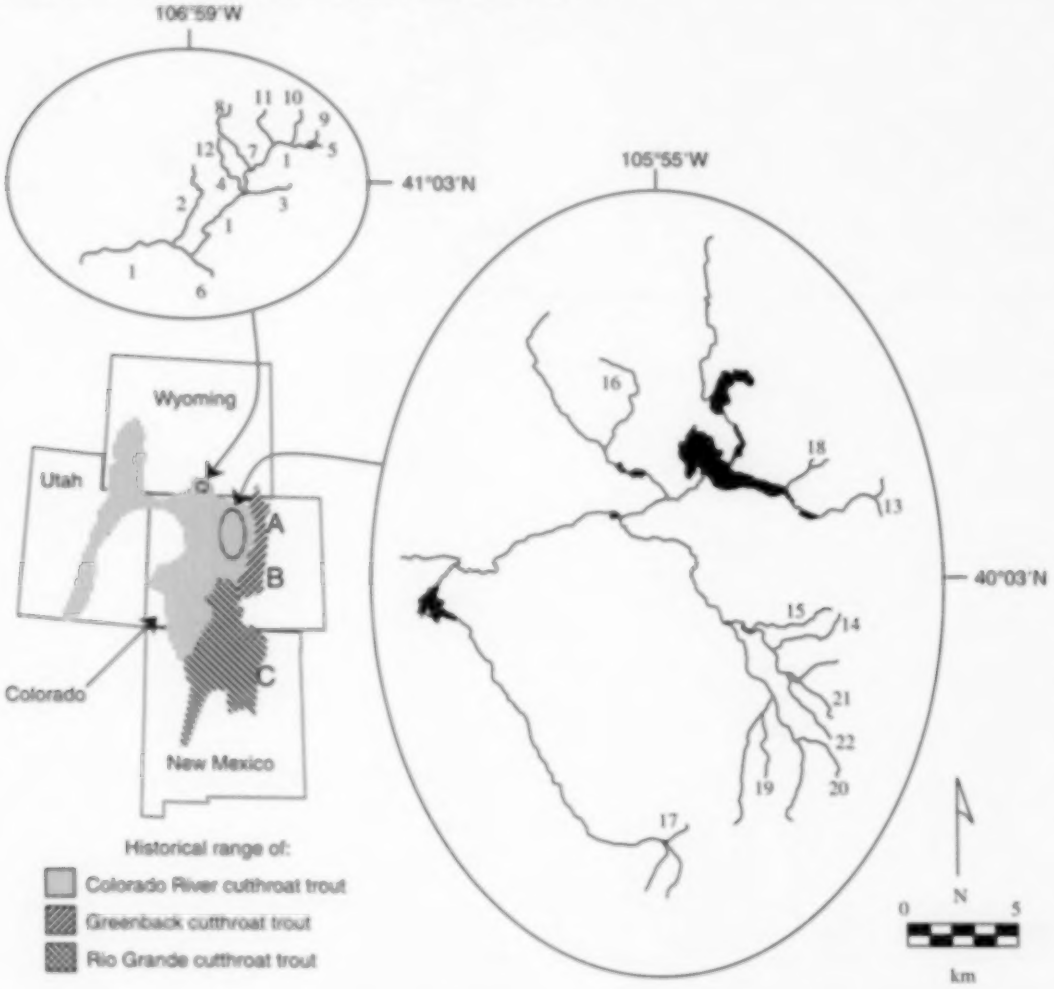
Fig. 1. Map of the US central Rocky Mountains showing 22 streams sampled to estimate cutthroat trout (*Oncorhynchus clarkii*) abundance, general locations of previous sampling, and historical ranges of Colorado River cutthroat trout (*Oncorhynchus clarkii pleuriticus*), greenback cutthroat trout (*Oncorhynchus clarkii stomias*), and Rio Grande cutthroat trout (*Oncorhynchus clarkii virginalis*). Circled areas denote locations of streams sampled in this study (refer to Table 1 for stream names associated with numbers). A, streams with greenback cutthroat trout sampled by Young and Guenther-Gloss (2004) and Harig and Fausch (2002) in the South Platte River basin; B, streams with greenback cutthroat trout sampled by Harig and Fausch (2002) in the Arkansas River basin; C, streams with Rio Grande cutthroat trout sampled by Harig and Fausch (2002).



ries with movements limited to a few kilometres (Young 1996). Cutthroat trout spawned after peak flows from late May to early July and fry typically emerged from August to September. Stream temperatures generally declined markedly by late September, with ice and snow cover often beginning to form in October. The short growing season produced slow-growing fish that rarely exceeded 250 mm total length.

Study design

Methods for estimating fish abundance followed Young and Guenther-Gloss (2004). We measured thalweg length of each stream with a drag tape, flagged 100-m intervals and electrofishing sites, and noted whether fish were present. We surveyed at least 500 m upstream of the last observation of fish or until a stream became largely uninhabitable or unlikely to support many fish (i.e., it was <0.5 m wetted width, above a waterfall, or originated from a spring). All sampling was conducted during late-summer low flows.

We used double sampling (Cochran 1977) to estimate abundance of cutthroat trout ≥75 mm total length (age 1 and older). Crews, usually consisting of one person electrofishing and two people netting, first made a single electrofishing pass without block nets in 25-m reaches at systematic intervals. Intervals between electrofishing reaches varied from 50 to 400 m to ensure an adequate number of samples in the shortest streams and resulted in sampling 5%–45% of each stream (Table 1). For second-stage samples, about every fourth sample reach was electrofished two or three times to produce a multiple-pass removal estimate of trout abundance. This sampling frequency ensured that at least four or

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Table 1. Characteristics of cutthroat trout (*Oncorhynchus clarkii*) streams in Wyoming and Colorado used in the models.

Stream	% of stream sampled	Trout abundance (≥75 mm)	Elevation (m)	Occupied stream length (m)	Pools ≥30 cm residual depth	Mean pool bankfull width (m)	Mean July water temperature (°C)
Colorado River cutthroat trout (*Oncorhynchus clarkii pleuriticus*), Little Snake River Basin, Wyoming							
1. NFLSR (lower)	10	4776	2252	14 545	385	7.5	9.7
2. Solomon	10	1705	2327	5 700	62	3.4	10.0
3. Green Timber	25	684	2523	3 150	83	3.3	9.3
4. Harrison (lower)	17	574	2518	2 557	66	2.8	9.7
5. NFLSR (upper)	17	378	2727	2 417	129	2.7	9.2
6. Rose	17	343	2365	1 670	33	3.5	10.1
7. Deadman (lower)	25	308	2584	1 322	45	4.4	9.6
8. Deadman (upper)	19	244	2740	3 175	116	3.4	8.0
9. Rhodine	23	177	2762	1 318	53	2.4	8.7
10. Tod (upper)	25	158	2724	1 940	53	2.7	8.5
11. Third (upper)	23	102	2720	1 315	45	3.0	10.3
12. Harrison (upper)	45	76	2760	553	12	2.0	8.5
Colorado River cutthroat trout), upper Colorado River Basin, Colorado							
13. Buchanan	15	2531	2774	5 725	63	6.5	10.8
14. Cabin	10	1354	2914	6 054	212	5.0	9.7
15. Hamilton	23	798	2865	3 225	46	2.1	8.5
16. Trail	10	594	2780	6 185	131	3.2	9.8
17. McQueary	21	379	3182	2 671	52	5.0	7.7
18. Roaring Fork	23	278	2731	4 164	160	4.9	9.0
19. Little Vasquez	18	115	2920	2 675	130	2.9	7.6
20. Jim	16	102	2853	2 225	10	3.9	7.2
21. Middle Fork Ranch	20	54	2896	2 395	28	4.8	7.7
22. South Fork Ranch	17	30	2939	2 880	26	3.0	8.2
Greenback cutthroat trout (*Oncorhynchus clarkii stomias*), South Platte River Basin, Colorado							
	5–21	170–7347	2323–2926	1 640–13 201	26–172	2.3–4.1	6.1–14.2
Greenback cutthroat trout and Rio Grande cutthroat trout (*Oncorhynchus clarkii virginalis*), Colorado and New Mexico							
	100	16–1278	2400–3293	1 000–20 500	12–361	2.3–5.4	6.0–14.6

Note: Numbers associated with stream names refer to the numbers in Fig. 1. Ranges for data on greenback cutthroat trout and Rio Grande cutthroat trout are from Harig and Fausch (2002) and Young and Guenther-Gloss (2004). NFLSR, North Fork Little Snake River. Percentage of stream sampled refers to the proportion of occupied stream length sampled for fish by electrofishing or visual methods.

five reaches would be sampled with multiple electrofishing passes in each stream in case calculating stream-specific catchability was required. In wider streams, two electrofishing crews worked side by side. To estimate population size of greenback cutthroat trout in Colorado and in tributaries containing Colorado River cutthroat trout in Wyoming, we multiplied the inverse of overall catchability for each set of streams by mean abundance of fish in single-pass catches in the sample reaches of a particular stream, extrapolated to the length of that stream (Bohlin et al. 1989). We deemed overall catchability appropriate because many of the same individuals (and the same crew leaders) formed these crews annually and they used the same equipment to sample these waters, and within each river basin, geology, habitat type, and riparian vegetation were comparable. Furthermore, we considered the stream-specific estimates based on fewer samples to be less likely to produce accurate estimates of population size in the set of comparable streams than would an estimate of overall catchability. In contrast, the abundance estimates for Colorado River cutthroat trout in Colorado were not collected in the same year and stream-to-stream variation in catchability was higher (M.K. Young, unpublished data); consequently, we used stream-specific catchability estimates to calculate abundance. We also used a separate estimator of catchability to calculate abundance of Colorado River cutthroat trout in the North Fork Little Snake River main stem in Wyoming because it was much larger and catchability was lower than for other streams in this area. All estimates for greenback cutthroat trout streams were based on samples collected in 1999, whereas those for Colorado River cutthroat trout represented the mean of four annual estimates from 1996 to 1999 (Wyoming) or one-time sampling between 1998 and 2002 (Colorado). We regard these population estimates as conservative because depletion electrofishing tends to underestimate abundance (Bohlin 1982; Peterson et al. 2004), although cutthroat trout in small streams typically yield high catchabilities (Dunham et al. 1999; Young and Guenther-Gloss 2004).

Habitat inventories varied by state. For Colorado streams, crews measured habitat in accordance with the methods of Harig and Fausch (2002). They recorded bankfull width, maximum depth, and tail crest depth for pools where pool length was at least half of bankfull width and censused the number of pools with residual depths of at least 30 cm. For streams in Wyoming, habitat components were only measured in electrofishing reaches (10%–45% of each stream); the means for reaches in a stream were extrapolated to the rest of that stream. We regarded such sampling as adequate

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because it greatly exceeded reach lengths (20–40 wetted or bankfull widths) generally used to describe geomorphic variables (Kaufmann et al. 1999). Also, only wetted width was measured, so we estimated pool bankfull width for Wyoming streams by multiplying pool wetted width by 1.5, the ratio between bankfull and baseflow wetted width based on measurements previously taken in two of the study streams (P.M. Guenther-Gloss, unpublished data).

Water temperatures were measured with thermographs (recording interval ≤2 h) set in relatively deep, well-mixed pools in Wyoming streams in 1997, 1998, and 2000 and in Colorado streams in 2001 and 2002. We used within- and among-stream and climatic comparisons from this 5-year period to develop a standardized mean July water temperature for each stream. Full-month mean July water temperatures were estimated for some Wyoming streams from the ratio of partial- to full-month means for nearby streams with complete July data. In Steelman Creek (Colorado), mean July stream temperature was estimated for 2001 by subtracting the mean difference for all other sampled Colorado streams (1.1 °C) from the 2002 temperature for Steelman Creek. We addressed the potential effect of climatic variation between years by examining annual deviation from the 92-year mean July air temperature at Steamboat Springs, Colorado (located in similar montane topography approximately equidistant between Colorado and Wyoming field sites; Western Regional Climate Center 2003). July air temperature in 1997 did not differ from the 92-year mean; all following years were warmer than average, with 2002 the warmest of all (3.3 °C above the mean). Unadjusted mean July stream temperatures also followed that pattern: all 10 Colorado streams were warmer in 2002 than in 2001, and 9 of 10 comparable Wyoming streams were warmer in 2000 than in 1997 or 1998. Thus, stream temperatures from 1998 to 2002 were adjusted downward using the ratio of study year to 92-year mean July air temperature to minimize effects of annual climatic variation on water temperatures used in the model. Finally, temperatures from multiple recording locations and multiple sampling years were averaged (in part to address other variables influencing water temperature, such as discharge) to provide a single temperature value for each stream.

Analyses

We treated fish abundance as a continuous dependent variable and assessed its relation to occupied stream length (m) and the three variables in the translocation model: mean July water temperature (°C), pool bankfull width (m), and number of pools with residual depths ≥30 cm. We included the latter because it was the simplest estimator of habitat availability and a necessary adjunct to the estimates of population size. Before developing models, we created a correlation matrix to assess collinearity among the physical variables and avoided simultaneous inclusion of those variables with significant correlations >0.60. We then used multiple linear regression to create models of all remaining possible subsets of the four explanatory variables ($N = 11$ possible models; see below). We examined residuals to detect outliers and heteroscedasticity and to evaluate overall model fit. Consequently, we applied a square root transformation to electrofishing-derived abundance estimates to correct for heteroscedasticity; visual counts required no such transformation. We selected a preferred model for each data set based on three criteria: weights (w) of Akaike's Information Criterion adjusted for small sample sizes (Burnham and Anderson 2002), all regression coefficients significantly different from zero, and ease of measurement of model variables. We considered all models with values of w within 10% of the best approximating model to have reasonable levels of support. To correct for multiple estimates of the significance of regression coefficients, we used a Bonferroni-corrected value of $P \leq 0.0125$ (=0.05/4, where 4 is the number of variables for which we generated estimates). Variables in order of their ease of measurement were water temperature = stream length → pool count = pool bankfull width. We deemed water temperature as one of the two easiest variables to measure because it could be obtained from only two site visits, to set and retrieve thermographs, although measurements needed to be collected in different years to estimate mean values. Occupied stream length was considered comparably easy to obtain because although the entire occupied stream channel needed to be traversed (to measure channel length), detailed surveys were not necessary and a single visit would be adequate to estimate occupied habitat barring dramatic environmental changes. In contrast, counts of pools ≥30 cm residual depth and pool bankfull width could be derived only from whole-stream inventories or extrapolated estimates of measured habitat types. Because streams with electrofishing-based abundance estimates originated from three distinct locations (the South Platte, Colorado, and Little Snake River basins), we included location as a categorical variable in the final regression model to determine whether there was a significant location-level effect (Dunham and Vinyard 1997). We conducted additional validation of the electrofishing-based model by using a jackknife approach, i.e., excluding one observation, reconstructing the model with the 30 remaining observations, predicting the response of the excluded observation, and examining the correlation between predicted and observed abundances (Olden and Jackson 2000).

Finally, we calculated means and 95% inverse prediction intervals (Zar 1984) of stream length predicted to support various population sizes of age-1 and older cutthroat trout based on the preferred model for electrofishing data. This enabled direct comparisons with previous work linking trout abundance to stream size (Hilderbrand and Kershner 2000). We did not consider predictions appropriate for the preferred model for visual counts because such counts may produce unreliable estimates of fish abundance (Bozek and Rahel 1991; Young and Guenther-Gloss 2004). Unless otherwise stated, results of statistical tests were considered significant if $P \leq 0.05$.

Results

Because occupied stream length and number of deep pools were highly correlated ($r = 0.82$, $P < 0.001$ for electrofishing estimates; $r = 0.80$, $P < 0.001$ for visual counts), we did not include both variables in any model. Of the 11 remaining models for streams with electrofishing estimates, three models including combinations of stream length, water temperature, and pool bankfull width had the highest model

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Table 2. Model selection statistics associated with the four highest ranking models relating mean July water temperature, pool bankfull width, counts of pools ≥30 cm residual depth, and occupied stream length to estimates of fish abundance.

Variable(s)	ln(*L*)	*K*	AIC_c	Δ	*w*
Electrofishing counts, Colorado River cutthroat trout (*Oncorhynchus clarkii pleuriticus*) and greenback cutthroat trout (*Oncorhynchus clarkii stomias*)					
Temperature, stream length	−60.926	4	131.390	0.000	0.612
Stream length	−63.499	3	133.887	2.497	0.175
Temperature, pool width, stream length	−60.882	5	134.165	2.775	0.153
Visual counts, greenback cutthroat trout and Rio Grande cutthroat trout (*Oncorhynchus clarkii virginalis*)					
Temperature, pool count	−103.150	4	216.800	0.000	0.794
Temperature, pool width, pool count	−103.002	5	220.003	3.203	0.160

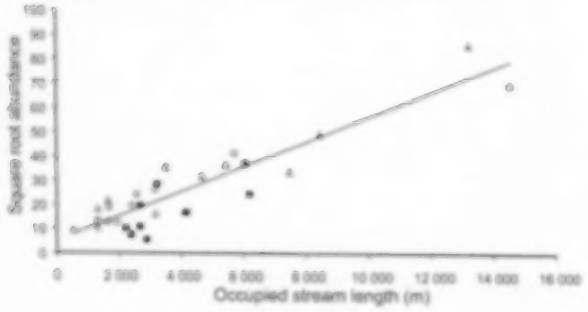
Note: Models are ordered by *w*. ln(*L*), maximized log-likelihood; K, number of parameters; AIC_c, Akaike's Information Criterion adjusted for small sample size; Δ, change in AIC_c; *w*, Akaike weight.

Table 3. Coefficients of variables of the highest ranking models relating mean July water temperature, pool bankfull width, sum of pools ≥30 cm residual depth, and occupied stream length to estimates of fish abundance.

Constant	Mean July water temperature (°C)	Mean pool bankfull width (m)	Pools ≥30 cm residual depth	Occupied stream length (m)
Electrofishing counts, Colorado River cutthroat trout (*Oncorhynchus clarkii pleuriticus*) and greenback cutthroat trout (*Oncorhynchus clarkii stomias*)				
−13.58	2.26*			0.00464*
5.15*				0.00508*
−12.03	2.20*	−0.37		0.00472*
Visual counts, greenback cutthroat trout and Rio Grande cutthroat trout (*Oncorhynchus clarkii virginalis*)				
−820.69*	91.39*		2.32*	
−903.97*	92.54*	20.74	2.30*	

Note: Models are ordered as in Table 2. An asterisk denotes that a coefficient is statistically different from zero at $P \leq 0.0125$.

Fig. 2. Relation of abundances of Colorado River cutthroat trout (*Oncorhynchus clarkii pleuriticus*) in Colorado (squares) and Wyoming (circles) and greenback cutthroat trout (*Oncorhynchus clarkii stomias*) in Colorado (triangles) to occupied stream length. The line represents the regression model (population size)$^{1/2}$ = 0.00508(occupied stream length (m)) + 5.148.



weights (Table 2). Pool bankfull width was not significantly different from zero (Table 3), so the model containing this variable was not considered further. The model including temperature and stream length had the highest model weight but required more intensive data collection from several years. Despite that it did not have the highest model weight, we regarded the model with stream length alone (Fig. 2) as the preferred model because it was the most parsimonious, required the least effort for data collection, and accounted for a large proportion of the variation in trout abundance (r^2 = 0.81). Also, differences in predicted abundances between the preferred model and best approximating model were small (median = 81 fish, range = 3–789 fish). For streams with visual counts, the best approximating model included stream temperature and number of deep pools as variables. The only other model with a reasonable level of support included those two variables and pool bankfull width. Because the latter variable was again not significantly different from zero, we regarded the best approximating model as the preferred model for visual counts.

Dummy regression with stream location and occupied stream length in a model using electrofishing estimates indicated that stream location did not substantially affect the model (i.e., neither the location coefficient (P = 0.43) nor location × length coefficient (P = 0.95) was significantly different from zero); thus, pooling locations in the preferred model was acceptable (Fig. 2). Jackknife validation suggested that the preferred model for electrofishing-based data was very precise (r = 0.88) but biased (slope coefficient = 0.80, 95% confidence interval = 0.63–0.97).

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Table 4. Means and 95% inverse prediction intervals of stream length predicted to support particular population sizes of age-1 and older cutthroat trout (*Oncorhynchus clarkii*) and stream length predictions from Hilderbrand and Kershner (2000) assuming a density of 200 fish·km^{-1}.

		95% prediction interval		
Population size	Predicted stream length (m)	Lower bound	Upper bound	Predicted stream length (m) (200 fish·km^{-1})
500	3 387	2 923	3 800	2 500
1000	5 209	4 783	5 710	5 000
2000	7 787	7 107	8 718	10 000
2500	8 825	8 008	9 965	12 500
5000	12 901	11 497	14 907	25 000

Projections of stream length needed to produce populations of 500–5000 age-1 and older cutthroat trout reflected the form of the relation between abundance and occupied stream length (Table 4). Assuming a uniform density of 200 fish·km^{-1} (as in Hilderbrand and Kershner 2000) slightly underestimated necessary stream lengths for small populations but greatly overestimated habitat needed to produce the largest populations.

Discussion

In the streams that we evaluated, occupied stream length was the single best predictor of cutthroat trout abundance. Bradford et al. (1997) reported a similar pattern for coho salmon (*Oncorhynchus kisutch*) smolts in western North America. Other studies have demonstrated that basin area, arguably a surrogate of stream length, is highly correlated with the presence or size of salmonid populations (Kruse 1998; Dunham and Rieman 1999; Dunham et al. 2002). Harig and Fausch (2002) noted that basin area was the only landscape-scale variable correlated with translocation success of cutthroat trout but offered little predictive power. Occupied stream length, however, is a more accurate measure of habitat availability than basin area (Dunham et al. 2002) because it accounts for the presence of natural barriers that constrain distributions of fish populations. Such barriers commonly limited the upstream and downstream extent of the allopatric populations that we sampled and defined habitat patches supporting discrete populations. Not addressed by our models were possible barriers within the occupied patches. Whether these were current obstructions to upstream migration could not be ascertained because allopatric populations of cutthroat trout occupied the areas upstream and downstream. Within-patch barriers would create subpopulations in a stream, with the upstreammost segment isolated from patches downstream and at greater risk of extinction from extreme disturbance (Dunham et al. 1999; Kruse et al. 1997). Data on emigration and immigration rates in a more advanced model would enable these streams to be evaluated as a series of partially isolated segments rather than as the cumulative total of occupied habitat.

We attribute the contrast in the response of fish abundance in the different models, as a function of the square of occupied stream length in the electrofishing-based model and as a linear function of water temperature and deep pool counts in the model for visual counts, to four factors. First, we suspect that colder streams probably diminished trout activity and detectability by observers (Thurow 1994). Second, efficiency of visual sampling probably declined as stream size and depth increased (Heggenes et al. 1990), and Young and Guenther-Gloss (2004) noted that visual counts ranged from 1% to 24% of electrofishing estimates and were only weakly correlated with them. Third, the absence of deep pool counts from the preferred model for electrofishing data implied that deep pools accounted for habitat availability less well than a more generic measure of available habitat or that rules for defining deep pools (i.e., that they be at least half as long as the bankfull width and at least 30 cm residual depth) excluded much suitable habitat. Alternatively, this variable may have been important in the original translocation model because trout are more easily observed in deep pools rather than in more turbulent or shallower sites, a bias much less evident in electrofishing data (Heggenes et al. 1990). Fourth, Bradford et al. (1997) noted that streams must be fully seeded for the habitat size – fish abundance relation to hold. Most streams that we sampled had supported cutthroat trout populations for decades and had little angling mortality; thus, populations may have approached the carrying capacity of the habitat, whereas Harig and Fausch (2002) examined populations of more recent origin in which this relation would be less certain. Also, streams with marginally successful translocations could be markedly influenced by small differences in pool numbers, width, or temperature, and some unsuitable patches may contain populations whose long-term persistence is unlikely (Morita and Yamamoto 2002). Consequently, we cannot regard the model based on visual counts as validating the model based on electrofishing estimates but believe that this discrepancy arose primarily from differences in the sampling methodologies used and streams chosen rather than shortcomings of the electrofishing-based model.

The failure of pool bankfull width to be included in either preferred model or to be significantly related to fish abundance when considered alone, despite that longer and presumably wider streams contained greater densities of fish, may have resulted from where bankfull width was measured. Pool bankfull width is a function not only of stream size but also of pool type, and because plunge, dammed, lateral scour, and trench pools have unique morphologies, variation attributable to pool type could overwhelm trends in width associated with stream size. Traditionally, measurements of bankfull width have been obtained in riffles or other channel units of fairly homogeneous width (Rosgen 1996), and we suspect that more consistent measures of overall stream

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width that enabled estimates of stream area may have been included in a preferred model and improved its performance.

Alternatively, because abundance increased in proportion to the square of occupied stream length in the electrofishing-based model, this relation may reflect increases in both habitat availability and diversity. Not only is supplemental habitat present in longer streams, but they are more likely to contain a complete suite of complementary habitats (e.g., temperature refugia, off-channel ponds, overwintering pools, summer rearing sites, or spawning areas suitable at varying flows) (Rieman and McIntyre 1995; Schlosser and Angermeier 1995). The lack of upstream–downstream trends in cutthroat trout density in these and other Rocky Mountain streams (Young and Guenther-Gloss 2004; M.K. Young, unpublished data) implies that habitat complexity contributes to the functional response between abundance and stream length. Also, temporal variation in habitat availability and the magnitude of disturbance are more pronounced in smaller streams and may influence this pattern.

The square root transformation of electrofishing estimates of fish abundance was necessary because the variance about the estimates increased with increasing stream size, producing a wedge-shaped distribution of points (Terrell et al. 1996). Theoretically, quantile regression on the uppermost distribution of points might suggest maximum carrying capacities that could be produced by streams of a given length (Dunham et al. 2002). Yet because none of the other habitat variables that we examined were included in the final regression model, we can offer little guidance on what habitat elements might be suppressing fish abundance in streams below such a regression line. This variation may also have arisen in part from the collection of abundance data in different years. Regardless, it seems more reasonable for rare subspecies of cutthroat trout to estimate average, rather than maximum, fish abundances when evaluating streams for introductions or the viability of existing populations.

To address issues of model precision and generality (Fausch et al. 1988), we tried to ensure that streams from different basins shared similar environmental characteristics and that sample sizes were adequate. Although cross-validation of the electrofishing-based model demonstrates that it is fairly accurate, the inherent bias (probably introduced by the square root transformation of abundance) limits its generality. Therefore, applications of the preferred model for electrofishing estimates should be restricted to systems comparable to those that we evaluated: small, high-elevation streams warm enough to permit recruitment. This model does not apply where high water temperatures (>22 °C) limit survival and growth (Dunham et al. 2003; Schrank et al. 2003). The streams used to develop the preferred model also were relatively free of human-associated disturbance, other than summer water withdrawals in some systems. Land management has often been associated with the decline in or absence of salmonid populations (Poff and Huryn 1998; Dunham and Rieman 1999; Peiss et al. 2002); thus, populations in altered systems may not conform to model predictions. In addition, we urge care in applying the model to streams subsidized by lake populations or interrupted by large numbers of beaver ponds. Although we included one such stream, McQueary Creek, in our sampling, a headwater lake possibly inflated the abundance and certainly the size of cutthroat trout; four of the eight largest trout that we sampled overall were in the vicinity of the lake. Finally, we suggest caution in applying the model to other subspecies. For example, whereas occupied stream length was significantly positively correlated ($r^2 = 0.71$, $P < 0.001$, $N = 19$) with the square root of abundance of allopatric Yellowstone cutthroat trout (*Oncorhynchus clarkii bouvieri*) in streams in the Bighorn River basin in Wyoming (Kruse et al. 2001, p. 6), the slope coefficient ($b = 0.00259$) was about half that ($b = 0.00508$) in the equation for streams in the central Rocky Mountains. That abundance still increased as the square of occupied stream length suggests that this functional relation may have some generality and merits testing with other subspecies in small, cold streams.

A variety of sources have hypothesized that persistence of trout populations, particularly with respect to long-term evolutionary potential, is related to abundances of 500–5000 fish (McIntyre and Rieman 1995; Allendorf et al. 1997; Hilderbrand and Kershner 2000). Our estimates of mean stream lengths necessary to produce populations of these sizes differed from those of Hilderbrand and Kershner (2000) because they assumed constant cutthroat trout densities of 100–300 fish·km^{-1}, whereas we found that trout density was positively correlated with occupied stream length and ranged from 10 to 557 fish·km^{-1}. Nevertheless, both analyses imply that many streams containing cutthroat trout support populations well below thresholds thought to afford resilience to environmental perturbation and loss of genetic variation (Allendorf et al. 1997; McElhany et al. 2000). For example, mean length of all recovery streams for greenback cutthroat trout was 4790 m (Young et al. 2002), for which we would predict a population size of 870 fish ≥75 mm (95% confidence interval = 701–1057 fish). Similarly, 40% of streams constituting the core conservation populations for Colorado River cutthroat trout in Colorado, Utah, and Wyoming are no more than 3000 m long (US Fish and Wildlife Service 2004); on average streams of this length would be predicted to contain 416 or fewer fish (95% confidence interval = 298–554 fish). Encouragingly, the preferred model demonstrated that relatively minor increases in habitat length led to disproportionately greater increases in abundance, implying that extending available habitat, particularly the downstream end, might be a key strategy for enhancing population viability.

Selecting streams for reintroductions and evaluating viability of existing populations of rare subspecies of cutthroat trout are politically controversial, biologically critical, and fraught with uncertainty (Young and Harig 2001). We believe that use of the preferred model for electrofishing data can reduce the ambiguity associated with predicting potential average size of reintroduced populations or mean size of unsampled populations, with two additional caveats. First, although map-derived estimates of potential occupied stream length may be a useful first approximation, the importance of barriers in structuring populations mandates that lengths be verified by field surveys. Second, there is substantial support that mean July water temperature was positively related to fish abundance in models based on electrofishing estimates and visual counts, probably reflecting increases in productivity at warmer temperatures. More importantly, because low water temperatures (mean summer water tempera-

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ture <7.5 °C) can inhibit cutthroat trout recruitment (Harig and Fausch 2002; Peterson 2002; M. Coleman and K.D. Fausch, Department of Fishery and Wildlife Biology, Colorado State University, Fort Collins, CO 80523, USA, unpublished data), prudent management dictates that decisions on conservation priorities and habitat protection must also consider whether summer water temperatures surpass the threshold necessary for successful recruitment. A single year of data collection might be sufficient to demonstrate whether temperatures are likely to be sufficiently warm. Finally, in the event that estimates of mean July water temperature and stream length are available, use of the model containing both variables will provide slightly more accurate estimates of population size.

Acknowledgements

We thank many individuals for help in the field and G. Horstman, K. Rogers, and B. Atkinson (Colorado Division of Wildlife) and A. Harig (Trout Unlimited) for sharing data. We also thank J. Dunham, A. Harig, D. Schmetterling, K. Rogers, and an anonymous reviewer for their comments on earlier drafts of the manuscript and R. King for a thorough biometrical review. The US Forest Service through the Arapaho-Roosevelt National Forests, Medicine Bow-Routt National Forests, Rocky Mountain Research Station, Region 2 Fish Habitat Relationships Program, and the interregional Inland Cutthroat Trout Initiative provided funding for this work.

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When fish meet a trawling vessel: examining the behaviour of gadoids using a free-floating buoy and acoustic split-beam tracking

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Abstract: The reaction of individual gadoids to a bottom-trawling vessel has been observed in situ in the Barents Sea using a free-floating buoy and acoustic target-tracking methods. More than 20 000 tracks were analysed in terms of velocity changes in vertical, athwarthship, and alongship direction relative to the vessel, the warps, and the trawl, respectively. The fish starts diving about 15 min before vessel passing. This coincides with the time the trawl is running and not with the gradual increase in vessel noise caused by the approaching vessel. The change in horizontal movement is more gradual and is directed away from the vessel in the alongship direction, but towards the vessel in the athwarthship direction. The strongest and sharpest response is related to the trawl warps. A strong herding in front of the warps is seen. Closer to the bottom, an athwarthship herding reaction is seen away from the trawl doors or possibly the lower parts of the warps. There were only minor differences when grouping the tracks according to light level, fish size, and fish density.

Résumé : L'utilisation d'une bouée dérivante et de méthodes acoustiques de suivi de cibles dans la mer de Barents a permis d'observer in situ la réaction de gadoïdes individuels à un chalutier de fond. Nous avons ainsi analysé plus de 20 000 trajectoires en ce qui a trait aux changements de vitesse en directions verticale, perpendiculaire et parallèle au navire, aux aussières et au chalut. Le poisson commence à plonger environ 15 min avant le passage du navire. Cela coïncide avec le temps d'utilisation du chalut et non avec l'accroissement graduel du bruit causé par l'approche du navire. Le changement dans le déplacement horizontal est plus graduel et il est orienté en direction opposée au navire chez les poissons qui se déplacent parallèlement au navire et en direction du navire chez les poissons qui se déplacent perpendiculairement au navire. Les réactions les plus fortes et les plus marquées sont associées aux aussières du chalut. On observe de forts rassemblements devant les aussières. Plus près du fond, il se fait une réaction de rassemblement vers le navire pour fuir les portes du chalut ou peut-être les parties plus basses des aussières. Il n'y a que des différences mineures lorsqu'on regroupe les trajectoires en fonction du niveau de lumière, de la taille des poissons et de la densité des poissons.

[Traduit par la Rédaction]

Introduction

When demersal fish encounter a trawling vessel, it is generally acknowledged that the fish (Ona and Godø 1990) dive towards the bottom and that they may disperse horizontally. But very little is known about the details of this movement. The aim of this paper is to quantify the reaction in terms of changes in velocity components of individual fish and to relate these changes in behaviour to the different parts of the vessel and gear. More precisely, we would like to pinpoint the ranges and times at which the different segments of the reaction start and end and discuss to what extent the response is caused by stimuli such as the vessel noise, the warps, and the trawl itself. For example, is the fish reaction primarily explained by the gradual increase in vessel noise caused by the approaching vessel, or are other factors of importance? How is the reaction towards the warps and trawl doors compared with the vessel itself? Is the response directional? Is it true that the fish is primarily herded away from the vessel? This kind of quantification of fish behaviour serves a double purpose: it constitutes a framework for understanding how the fish detects and reacts to the approaching vessel, and it is important for interpreting trawl survey data on their own and in combination with acoustic information.

To achieve this goal, we collected noise data on the vessel and its gear, and the reaction of more than 20 000 fish have been monitored individually by acoustic tracking employing a split-beam echo sounder. The velocity of each fish has been decomposed in a vertical component, an alongship component, and an athwarthship component, enabling us to quantify possible avoidance–herding phenomena in three dimensions. Moreover, by using coordinate systems centred at the propeller, the warps, and the trawl doors, one can compare the strengths of directional responses due to each of these potential triggers of behavioural changes in fish.

Earlier studies of fish response towards approaching trawling vessels have mainly been based on aggregated data, such as acoustic echograms. Apparently not much progress has been made since the review by Godø (1994) a decade ago. The findings that demersal fish typically respond by swimming towards

Received 6 July 2004. Accepted 23 March 2005. Published on the NRC Research Press Web site at http://cjfas.nrc.ca/ on 24 September 2005.
J18206

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 doi: 10.1139/F05-131 © 2005 NRC Canada

the bottom (Ona and Godø 1990; Nunnallee 1991) are based on interpretations of density echograms recorded from a buoy or another vessel in the path of the approaching trawler. In addition, Ona and Godø (1990) compared the echo-integrated fish density while trawling with that in adjacent areas when cruising at normal acoustic-survey speed. They found that the echo density decreased during trawling at depths between 0 and 200 m. However, recent investigations have disputed these results to some extent (V. Hjellvik, Combining Acoustic and Trawl data for Estimating Fish Abundance (CATEFA) Project, Institute of Marine Research, P.O. Box 1870, 5817 Bergen, Norway, unpublished data).

Similar experiments have been performed for free-running vessels during acoustic surveying (i.e., without trawl). The reactions of Atlantic herring (*Clupea harengus*), Atlantic cod (*Gadus morhua*), capelin (*Mallotus villosus*), and Arctic cod (*Boreogadus saida*) were investigated by Olsen et al. (1983). More recently experiments were repeated for herring (Vabø et al. 2002). Olsen (1990) pointed out several sources of error in acoustic abundance estimates induced by fish behaviour. Soria et al. (1996) investigated the reaction of fish schools by comparing the results from a multibeam sonar with a vertical echo sounder. More recently, Fernandes et al. (2000*a*, 2000*b*) reported that fish did not avoid the noise-reduced research vessel *Scotia* by comparing the acoustic density determined by an autonomous underwater vehicle running ahead of the survey vessel with that from the vessel itself.

The methods used in these previous investigations work if the difference between true biomass and measured biomass can be established. Based on this information, inferences may be drawn about the vertical and horizontal movement of fish aggregations. However, the methods give no behavioural information in terms of velocity changes. This requires the tracking of individual fish, as done in the present paper and for which very few results are available. Engås et al. (1998) did track fish with a radio acoustic positioning system, but the sample sizes were only 13 cod. Furthermore, some preliminary results were published in Handegard et al. (2003), where a vertical diving response, consistent with Ona and Godø (1990), and a general increase in horizontal speed were found.

Materials and methods

The experiments

The R/V *G.O. Sars*, built in 1970, was used in the experiments. The vessel had 24-mm, 2.09 kg·m^{-1} trawl warps (wires) produced by ScanRope (Tønsberg, Norway), a Campelen 1800 shrimp trawl (Campelen, Bergen, Norway), and rockhopper gear. The trawl was equipped with a Simrad (Kongsberg Gruppen, Kongsberg, Norway) integrated trawl instrumentation (ITI) system mounted at the trawl mouth to estimate the position of the trawl relative to the vessel. A description of the ITI system is given in Engås et al. (2000). The position of the vessel was determined using the onboard global positioning system (GPS). The echo sounder provided no species information, so pelagic trawl samples using an Åkra trawl were taken in addition to the bottom trawl catches for species identification. The caught fish were mainly gadoids (Table 1), and the catches were composed of 51% (in numbers) cod, 18% haddock (*Melanogrammus aeglefinus*), and 15% saithe (pollock) (*Pollachius virens*). In addition there were 16% redfish (*Sebastes* sp.). Since cod dominated the catches and recognizing the potential bias due to varying catchability for the species involved, we assumed that the general behaviour pattern seen in our investigations can be attributed to cod.

The experiments were conducted off the coast of Finnmark (71°N, 24°–31°E) in March 2001 and April 2002. This is when immature cod prey upon capelin migrating to the shores of Finnmark for spawning, and the mature cod are spawning in the Lofoten area (Bergstad et al. 1987). The time of year, the trawl, and the species composition are representative of the Norwegian winter bottom trawl survey in the Barents Sea (Jakobsen et al. 1997). The bottom depth varied from 140 to 300 m, with a median of 290 m.

The fish reactions were measured by deploying a free-floating buoy (Bergen Acoustic Buoy; refer to Godøet al. 1999) containing a split-beam echo sounder. The rigging of the buoy and the passings were conducted as described in Handegard (2004, pp. 87–90). This buoy has a GPS, and the transducer housing contains a compass. Successive passings by the trawler were made while tracking individual fish with the buoy echo sounder. A total of 54 buoy passings were conducted. The 2001 experiments included 16 passings over seven deployments of the buoy, while in 2002, there were 38 passings over eight deployments of the buoy. In total, there were 16 daytime passings, 30 nighttime passings, and 8 twilight passings. The minimum time between passings was 50 min, the median within each deployment of the buoy being 120 min. The transducer was deployed below the buoy to avoid vessel-induced pendulum movement. The buoy–transducer depth varied between 37 and 47 m, with a median of 44 m. Wind and ocean waves can induce buoy movement, possibly affecting the transducer tilt–roll angles. To reduce this unwanted effect, we used a special buoy setup where weights and floats were used to stabilize the transducer during operation (Handegard et al. 2003, their Fig. 1).

Noise measurements of *G.O. Sars* were conducted in Stjernøyfjorden (70°13′N, 22°48′E) on 18 March 2001. A lifeboat was launched with two Brüel & Kjær (Nærum, Denmark) hydrophones (model 8106) deployed at 60 and 20 m depth. The hydrophones were connected to a Brüel & Kjær dual-channel real-time frequency analyser (model 2144) through a microphone pre-amplifier (model 5935) and type AC0101 cables, and a Sony TCD-D1 Pro-Dat tape recorder (Sony Corp., Tokyo, Japan) was used to record the data. Three passings were conducted, and the total integrated noise level in the range 40–400 Hz was calculated (Fig. 1). Events during boat handling and the start of trawling were registered, and these are indicated in the same figure. The trawl warp vibrations were measured in both passings using an accelerometer on the trawl wires. The spectrograms were obtained from the frequency analyser. Two main spectral peaks at 7 and 14 Hz were found.

Obtaining the tracks

The Simrad EK60 Scientific Echosounder (Kongsberg Gruppen) single echo detection (SED) algorithm was used to estimate the position of individual fish within the echo beam. The minimum and maximum echo length was set to 0.8 and 1.8 times

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Table 1. Trawl catches in weight (W) and number (N) per nautical mile by species.

Date	Time	Station[a]	Light level[b]	Pos[c]	Cod[d]		Haddock[e]		Saithe[f]		Redfish[g]	
					W	N	W	N	W	N	W	N
March 2001												
14	0150			1	376	181	14	70	3	1	4	3
14	0757	a		0	350	195	22	107	78	59	24	30
14	1404		D	1	468	269	31	37	212	145	12	13
14	1643		N	1	535	251	40	81	10	10	5	5
14	1906	b	N	1	0	0	0	0	0	0	0	0
14	2050		N	0	119	59	8	35	11	0	6	5
14	2210		N	0	58	30	0	5	2	2	3	3
15	0030		N	1	103	53	12	58	14	11	8	8
15	0224		N	1	116	49	18	40	21	18	2	2
15	1945		N	1	150	87	27	57	17	14	4	7
15	2120		N	1	101	52	21	118	20	15	5	4
16	1903	b	N	1	0	0	0	0	0	0	0	0
16	2110	b	N	1	0	0	0	0	0	0	0	0
16	2336	a	T	0	1050	609	21	15	3	2	2	2
17	1037	b	D	1	0	0	0	0	0	0	0	0
17	1235	a	T	0	45	17	5	3	1	1	0	0
17	1807		N	1	247	126	25	25	5	4	4	4
17	2123		N	1	328	143	15	43	59	53	4	4
17	2212		N	1	229	133	22	19	23	21	18	20
April 2002												
8	1703		D	0	122	76	3	11	29	13	0	1
8	2149		N	1	1313	563	0	0	242	156	1061	1138
9	0007	b	T	1	0	0	0	0	0	0	0	0
9	0215	b	D	1	0	0	0	0	0	0	0	0
9	0426	a	T	0	643	242	30	32	4	4	0	0
9	1305		D	1	1176	889	92	174	105	88	882	1221
9	1526	b	D	1	0	0	0	0	0	0	0	0
9	1717	b	T	1	0	0	0	0	0	0	0	0
9	1844	b	T	0	0	0	0	0	0	0	0	0
9	2000	b	N	1	0	0	0	0	0	0	0	0
9	2133	b	N	0	0	0	0	0	0	0	0	0
9	2311		N	1	504	312	3	42	4	4	6	6
10	0051	b	N	1	0	0	0	0	0	0	0	0
10	0219	a	T	0	79	29	10	12	0	0	0	0
10	1357	a	T	0	142	74	55	70	4	4	0	0
10	1631	b	D	0	0	0	0	0	0	0	0	0
10	1742	b	T	1	0	0	0	0	0	0	0	0
10	1852	b	T	0	0	0	0	0	0	0	0	0
10	2023		N	1	871	553	48	123	66	52	135	148
10	2151	b	N	1	0	0	0	0	0	0	0	0
10	2338	a	T	0	37	20	15	21	0	0	0	0
12	2040		N	0	498	402	80	330	1200	612	194	246
12	2352	b	N	0	0	0	0	0	0	0	0	0
13	0034	a	T	0	16	9	2	4	1	1	0	0
13	0254	b	N	1	0	0	0	0	0	0	0	0
13	0523	b	D	1	0	0	0	0	0	0	0	0
13	0658		D	1	826	609	75	554	273	154	225	215
13	2035		N	0	122	91	1	14	13	7	1	3
13	2242		N	1	131	93	0	0	2	1	2	3
14	0035	a	T	0	7	3	6	26	0	0	0	0
14	0225		D	0	246	174	0	0	0	0	5	4

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Table 1. (*concluded*)

Date	Time	Station[e]	Light level[f]	Pos[g]	Cod[a]		Haddock[b]		Saithe[c]		Redfish[d]	
					W	N	W	N	W	N	W	N
April 2002												
14	4:54		D	0	212	130	2	107	0	0	4	3
14	7:02		D	1	360	242	0	0	9	4	6	6
14	8:13		D	1	225	152	15	459	6	0	7	7
14	18:47		T	1	792	609	134	236	62	86	0	0
14	20:09	b	N	1	0	0	0	0	0	0	0	0
14	22:06		N	1	1530	671	21	29	18	7	0	0
14	23:46	a	T	0	231	126	13	16	17	19	0	0
15	2:13	b	D	1	0	0	0	0	0	0	0	0
15	3:21	b	D	1	0	0	0	0	0	0	0	0
15	18:26		T	1	1765	1605	365	599	1367	1356	10	23
15	20:15	b	N	1	0	0	0	0	0	0	0	0
15	21:46	b	N	0	0	0	0	0	0	0	0	0
15	23:11	b	N	1	0	0	0	0	0	0	0	0
16	0:23	b	T	1	0	0	0	0	0	0	0	0
16	2:55	b	D	1	0	0	0	0	0	0	0	0
Total					16 122	9928	1253	3569	3893	2925	2640	3133
Proportion (%)					67	51	5	18	16	15	11	16

[a]*Gadus morhua.*
[b]*Melanogrammus aeglefinus.*
[c]*Pollachius virens.*
[d]*Sebastes* sp.
[e]Pelagic stations for species identification are denoted "a". These are used for species identification in the upper water column and do not count as vessel–buoy passings. Bottom trawl stations with open cod end are denoted "b".
[f]Three classes of light levels: D, day; N, night; and T, twilight.
[g]Pos, position. 1 indicates that the integrated trawl instrumentation (ITI) were functioning and were consistent with detections of the warp on the echograms. 0 indicates that the ITI was not functioning or that the warp detections on the echogram were inconsistent with the ITI trawl position estimates.

the pulse length, respectively, and the maximum phase deviation and the maximum gain compensation was set to 8.0 phase steps and 6.0 dB, respectively. Next, these targets were composed into tracks using a target-tracking algorithm designed for fish observed from a moving platform (Handegard et al. 2005). This algorithm determines the correlated behaviour of targets within the echo beam and uses this to estimate the tilt–roll–heave orientation of the transducer.

The warps produce signals that could easily be misinterpreted as fish echoes. To avoid any interference with the analysis of fish behaviour, we manually removed registrations that could be interpreted as warps in every passing. The tracking is performed in a geo-referenced coordinate system, where the buoy's GPS, the transducer compass, and the tilt–roll–heave estimates are used to position each track. Fitting a regression line

$$\hat{\boldsymbol{x}}^{(i)}(t_k) = \hat{\boldsymbol{s}}_i + [t_k - \bar{t}_i]\hat{\boldsymbol{u}}_i$$

through the connected single targets has proved to be a robust method of representing each track (Handegard et al. 2005). Here, $\hat{\boldsymbol{x}}^{(i)}(t_k)$ is the estimated 3-dimensional position at time t_k of track i, $\bar{t}_i$ is the mean time point for this track, and $\hat{\boldsymbol{s}}_i$ and $\hat{\boldsymbol{u}}_i$ are parameters to be estimated. The mean time point $\bar{t}_i$ is measured relative to the vessel transducer passing of the buoy, with positive time after the buoy passing. Note that $\hat{\boldsymbol{s}}_i$ is the estimated mean position of the track, which is used to position the track in the further analysis. The parameter $\hat{\boldsymbol{u}}_i$ can be interpreted as the average velocity vector of the track. The fitting is done by least squares (i.e., by minimizing the following equation):

$$\mathrm{SS} = \sum_k \|\tilde{\boldsymbol{x}}^{(i)}(t_k) - \hat{\boldsymbol{x}}^{(i)}(t_k)\|^2$$

where $\tilde{\boldsymbol{x}}^{(i)}(t_k)$ are the measured positions from the echo sounder, and $\|\cdot\|$ is the Euclidean norm with $\|\cdot\|^2 = \sum_{j=1}^{3} x_j^2$ for $\boldsymbol{x} = [x_1\, x_2\, x_3]$ (see e.g., Handegard et al. 2003, their Fig. 2). The end result of the regression analysis for track i is that it is represented by the position–velocity pair $(\hat{\boldsymbol{s}}_i, \hat{\boldsymbol{u}}_i)$. Although the pair is estimated, to simplify, we omit the hat notation from here on.

A vessel-oriented coordinate system is used in the further analysis. The x axis is defined as the horizontal projection of the straight line from the trawl mouth to the propeller. The positive z axis is pointing from the bottom to the surface. The coordinate system is right-handed and orthogonal. Note that when changing from the geo-referenced coordinate system to the vessel-oriented coordinate system, only the direction of $\boldsymbol{u}_i$ is altered (i.e., the vessel velocity component is not included in $\boldsymbol{u}_i$). In the following analysis, the index i denoting the track number is omitted, and $\boldsymbol{s}$ and $\boldsymbol{u}$ are given relative to the vessel-oriented coordinate system.

The positioning of the vessel and gear, and thus the alignment of the vessel-oriented coordinate system, were obtained using the data from the GPS on the vessel and the Simrad Trawl Instrumentation system (ITI) on the trawl. The ITI returned

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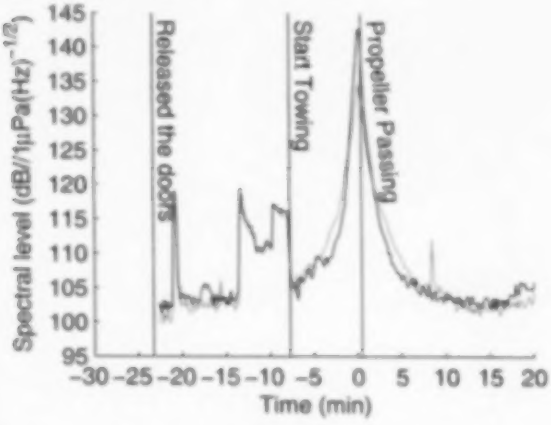
Fig. 1. Total integrated noise level between 40 and 400 Hz for the hydrophones at 20 and 40 m depth, shown as black and grey lines, respectively. Three passes were made, and this figure presents the result from the second passing. The two other passes were similar. Note that the sound levels are the absolute levels at 20 and 40 m depths, respectively, and not corrected for the distance to the vessel. Black vertical lines are events registered during the experiment.



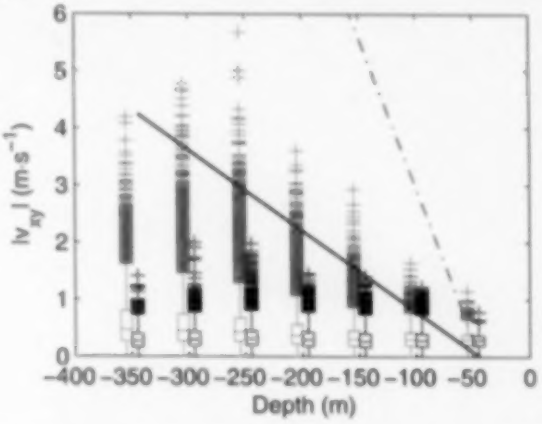
the angle and the distance to the trawl headline. In the case of failure of the ITI, the trawl was supposed to be in the path of the vessel. However, this assumption is not necessarily correct (Engås et al. 2000). The position error of each track relative to the geo-referenced coordinate system is approximately ± 40 m. The position error of the coordinate system due to an inaccurate trawl position increases with distance and is not quantified. However, a technique to verify the position estimates based on the buoy echo sounder registrations of the warps was used (Handegard 2004, pp. 87–90).

The tracks have been subjected to robustness tests involving estimated velocity, depth and current dependence, and speed of the vessel. Based on these tests, selection criteria were applied to the tracks, and those judged to be unacceptable were removed. To test the robustness of the estimated velocity, we compared the speed and horizontal direction of the original track with those of resampled subtracks. The median differences in horizontal speed and swimming angle between the resampled and original tracks were about 0.006m·s^{-1} and 10°, respectively.

The horizontal speed of a track depends on the depth, and this dependence was related to the track length. To estimate this relationship, we performed a regression of the horizontal speed $\sqrt{u_x^2 + u_y^2}$ against depth. Here u_x and u_y are x and y components of $\boldsymbol{u}$, respectively. The regression slope was recalculated after removing tracks shorter than a fixed minimum track length, thus obtaining the depth-dependent effect as a function of minimum track length. Based on this and the resampling tests, tracks shorter than 15 pings were removed. It is seen that the depth dependence is strongly reduced for the accepted tracks (Fig. 2). See the Discussion section for possible explanations of the depth effect.

When approaching the buoy, the distance and time before passing should be linearly related (i.e., the vessel should approach the buoy at constant speed). The time between the trawl

Fig. 2. The horizontal swimming speed $|v_{xy}|$ stratified in 50-m depth bins, showing increased swimming speed with depth. The boxes cover the 25th percentile, the median, and the 75th percentile, while the length of the whiskers are 1.5 times the distance between the 25th and 75th percentiles. Data points outside the whiskers are marked as +. The gray boxes show all data, while the black boxes are based on tracks with minimum track length >15 pings. The black solid line and the gray dash-dotted line show the maximum detectable linear swimming speed at a given depth assuming 1-s ping rate for the cases minimum track length >15 pings and minimum track length >2 pings, respectively.



running and the vessel passing of the buoy was variable. Often the distance and time were not linearly related (Fig. 3). Looking at the position $s_x = s_{x,i}$ of track i in the alongship direction, only those tracks with s_x in the interval

$$(-v - \Delta v) \cdot t_i + x_{\text{offset}} - \Delta x < s_x < (-v + \Delta v) \cdot t_i + x_{\text{offset}} + \Delta x$$

were accepted. Here, v is the nominal vessel speed, Δv is the accepted difference in vessel speed from v, t_i is the time before–after the buoy transducer passing for track i, x_{offset} is the distance between the transducer and the propeller, and Δx is the accepted error in the position estimate in the alongship direction (Fig. 3). The parameters were set to $v = 1.54$ m·s^{-1} (3 knots), $\Delta v = 0.51$ m·s^{-1} (1 knot), $x_{\text{offset}} = 70$ m, and $\Delta x = 100$ m.

The maximum target strength (TS) was calculated along each track. This is an approximate measure of fish size. Only tracks whose maximum TS was in the interval −45 to −20 dB were accepted. This corresponds to fish lengths above ~ 12 cm for cod (Nakken and Olsen 1977).

The measured track velocity is a combination of current drift and active swimming velocity. Unfortunately, water current measurements were not available, and a simple method to remove the current dependence was implemented. The depth-dependent mean horizontal swimming velocity from 30 to 15 min before each passing was used as an estimate of the current velocity (it is rather the mean velocity of the fish in the null situation of no vessel-related stimuli) for the passing. The depth dependence was found by binning the velocities into four depth groups. Since we are interested in fish reaction, these estimates were subtracted from the track velocities for each passing, and

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Fig. 3. The alongship position s_x for each track as a function of time t before vessel–buoy passing. The black lines define the area where the tracks are valid (i.e., where the vessel is approaching the buoy at constant velocity). The gray and black dots are, respectively, the accepted and rejected tracks.

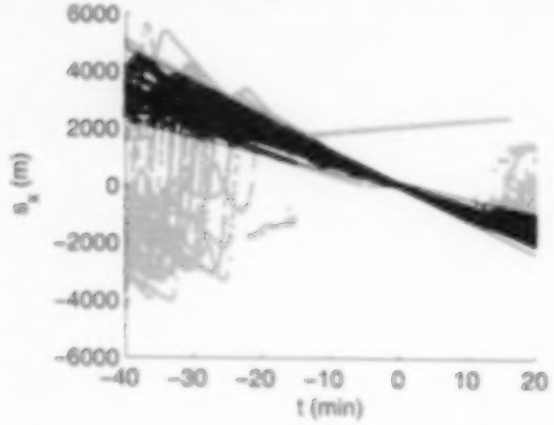


Table 2. Number of total, accepted, and rejected tracks.

All tracks	144 430
Outside path tracks	86 026
Invalid TS tracks	7 654
Short tracks	84 428
Sum of OK tracks	23 329

Note: TS, target strength.

the adjusted velocities were used in the subsequent analysis. The main analysis was based on these estimates, but as a check it was also carried through on the uncorrected data and on data adjusted by the mean null-hypothesis velocity (i.e., the velocity estimate without depth dependence).

Based on the quality screening described above, we finally kept 23 329 tracks (Table 2) with position–velocity coordinates $(\boldsymbol{s}, \boldsymbol{u})$, where the $\boldsymbol{u}$'s are corrected for water currents. In addition, we have the time $t = \bar{t}_i$, relative to vessel passing, for each track.

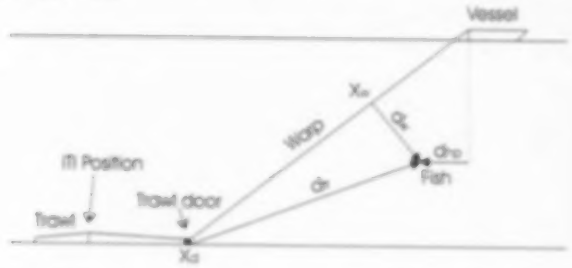
Response and explanatory variables

Several explanatory and response variables were derived from $\boldsymbol{s}$ and $\boldsymbol{u}$. They are based on the position $\boldsymbol{s} = [s_x\ s_y\ s_z]$ of the fish and its velocity $\boldsymbol{u} = [u_x\ u_y\ u_z]$, the estimated position of the trawl doors $\boldsymbol{x}_d$, the time relative to buoy–vessel passing t, and a separate time variable, t_{tow}, relative to the start-towing event, with $t_{tow} = 0$ at this event (Fig. 4).

A simple model was used to calculate the distance of each tracked fish from the trawl doors and the warps. A straight line of 60 m connects the ITI position to the doors, and one single straight line models the trawl warps (Fig. 4).

The explanatory variables are the horizontal distance to the propeller, the distance to the warps, the distance to the trawl doors, the time relative to start towing, and the time relative to buoy-vessel passing. The horizontal distance to the propeller is

Fig. 4. The vessel and gear model. The known integrated trawl instrumentation (ITI) and vessel positions are used to calculate the distance to the warp d_w, the distance to the trawl door d_d, and the horizontal distance to the propeller d_{hp}. Here, $\boldsymbol{x}_w$ is the point on the warp closest to the track, and $\boldsymbol{x}_d$ is the estimated position of the trawl door.



defined as

$$d_{hp} = \mathrm{sgn}(s_x)\sqrt{s_x^2 + s_y^2}$$

where sgn is the signum function (i.e., $\mathrm{sgn}(s_x) = +1$ for $s_x \geq 0$ and $\mathrm{sgn}(s_x) = -1$ for $s_x < 0$). This means that d_{hp} is positive before passing and negative after. The distance to the warp is

$$d_w = \|\boldsymbol{s} - \boldsymbol{x}_w\| \cdot \mathrm{sgn}(s_x - x_{wx})$$

where $\boldsymbol{x}_w$ is the point closest to $\boldsymbol{s}$ on the line between the estimated trawl door position and the propeller position, and x_{wx} is the x component of $\boldsymbol{x}_w$. The distance to the trawl doors is

$$d_d = \|\boldsymbol{s} - \boldsymbol{x}_d\| \cdot \mathrm{sgn}(s_x - x_{dx})$$

where x_{dx} is the x component of $\boldsymbol{x}_d$. The start-towing event is the instant when the trawl is running properly, and t_{tow} is the time variable relative to this event. This is routinely registered for all trawl hauls conducted by Institute of Marine Research (Bergen, Norway). The time of start towing ($t_{tow} = 0$) relative to the time the vessel passes the buoy ($t = 0$) varies between passings, and t_{tow} is thus not offset with a constant time relative to t. Finally, the time t before–after passing is used as an explanatory variable. Note that t is positive after the vessel passing, while the ds are negative.

The response variables are based on $\boldsymbol{u} = [u_x\ u_y\ u_z]$. To accommodate the need for an appropriate notation for the horizontal speed and the athwartship component, it is convenient at this point to change the symbols for velocity. The vertical velocity component is denoted $v_z = u_z$, the vertical speed $|v_z| = |u_z|$, and the horizontal speed $|v_{xy}| = \sqrt{u_x^2 + u_y^2}$. Note that speed has no direction, while velocity has. To test for any horizontal directional response, we used the alongship swimming component $v_x = u_x$, and the signed athwartship component $v_y = u_y \cdot \mathrm{sgn}(s_y)$. The signed athwartship component is positive when a fish is swimming away from the x axis and negative when swimming towards the x axis, whereas v_y is positive when a fish is swimming in the same direction as the vessel is moving. An overview of the explanatory and response variables is given (Table 3). All these variables are contained in one data file that includes the tracks from all passings.

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Table 3. The response and explanatory variables.

Response	
$\lvert v_z \rvert$	Vertical absolute speed (m·s^{-1})
$\lvert v_{xy} \rvert$	Horizontal absolute speed (m·s^{-1})
v_x	Alongship direction of u (component along x axis, positive along vessel heading)
v_y	Signed athwartship component of u (signed component along y axis, positive perpendicular away from vessel path)
v_z	Vertical component of u (component along z axis, negative when diving)
Explanatory	
d_{hp}	Horizontal distance propeller (m) (positive in front of the vessel)
d_w	Distance to warp (m) (positive in front of the warp)
d_d	Distance to trawl door (m) (positive in front of the vessel)
t	Time relative to vessel transducer passing (min) (negative before vessel passing)
t_{haw}	Time relative to the start trawling event (min) (negative before the start trawling event)

As potential additional explanatory variables, we have considered the light level at the sea surface, the maximum TS for each track, the mean integrated echo energy around each track, and a categorical variable indicating year 2001 or 2002. The surface light level was divided into the categories dark, light, or twilight; the diel variations were tested by analysing the dark and light groups separately. Tracks were binned into two size groups, above and below a TS threshold of $= -30$ dB, which corresponds to a cod approximately 50 cm long. The mean integrated echo energy for each track from 1 min before–after the first–last ping in the track and 10 m above–below the track was extracted. The summed energies were taken as a measure of the fish density. The data were binned into two groups, above and below the median of the integrated echo energy.

Statistical analysis

The first step of the statistical analysis was to calculate nonparametric fits between pairs of response (velocity) variables and explanatory (position–time) variables, all passings together, and then categorized on explanatory variables as defined above. Note here that modelling the response in terms of combination of variables was not instructive (see the Discussion), and the reaction is analysed in terms of each explanatory variable separately.

Two methods were used. One is a standard, nonparametric smoothing algorithm, such as the generalized additive model (GAM), to estimate the response as a function of the explanatory variables. We have used the smoothing-spline algorithm in the R program GAM module (R Development Core Team 2003). The degree of smoothing is determined using cross-validation, which results in a high degree of smoothing. The error intervals are fixed 95% confidence intervals for each value of d or t, not confidence bands.

On some occasions, the details of the response were smoothed out by the GAM. To reveal these details, we used a more or less direct extension of the method of Handegard et al. (2003), using a running mean (RM) with fixed nonoverlapping windows (Figs. 5*a*, 6*e*, and 6*f*). The estimated velocities were binned into groups referred to as running-mean windows. Outliers were eliminated by removing tracks whose variables exceeded three standard deviations. The bins themselves were nonoverlapping intervals of the explanatory variables. The steps in the time and distance variables were, respectively, $\Delta t = 1$ min and $\Delta d = 50$ m. This technique was also used for the 3D representation of the response (Fig. 7). Here the data is further divided into depth bins with steps of $\Delta z = 50$ m and distance bins with steps of $\Delta d = 100$ m.

The above-mentioned methods do not explicitly detect when the change in behaviour occurs. We are particularly interested in searching for values of the explanatory variables d or t associated with changes in the behavioural pattern. The statistical problem of finding such points of change is quite complex. In full generality, it involves piecing a series of curve segments together and assessing the presence and number of segment transitions. As a simplification, we have chosen to fit a piecewise linear function. For our data, this works well in most cases (see, e.g., Fig. 5). Several algorithms exist for fitting a series of linear regressions. We have adopted the procedure outlined in Muggeo (2003; see Appendix A). This idea goes back to Box and Tidwell (1962) and is also mentioned by McCullagh and Nelder (1989, p. 379). Muggeo (2003) has implemented an R routine "segmented", which we have adopted. The method requires a priori estimates of the breakpoints. These are determined by inspecting the nonparametric fits.

The R implementation of the segmented regression occasionally produces discontinuities in the curves (see e.g., the segmented regression line for $(t, \lvert v_{xy} \rvert)$ (Fig. 5b)). The curves are by definition continuous, and any gap between two consecutive fitted lines is not significant, being an artefact of the method. The gap may be assumed zero since the true lines in the underlying population are continuous.

Results

The results are presented in terms of three figures. The reaction in terms of t and the response variables gives a good overview of the larger-scale behaviour (Fig. 5), where the start is at $t = -30$ min, which corresponds to $d_{hp} \sim 900$ m. A selection of response variables as a function of distance to trawl doors and warp are presented (Fig. 6). The nonparametric spline, the fitted segmented regression lines, and the breakpoints with ±2 SEs are displayed on these figures, and the RM curve is included in the cases where references are made in the text (Figs. 5*a*, 6*e*, and 6*f*). Finally, a three-dimensional view of the reaction is given (Fig. 7).

The fish reaction starts comparatively far away in the prevessel zone (i.e., long before the vessel transducer passes the

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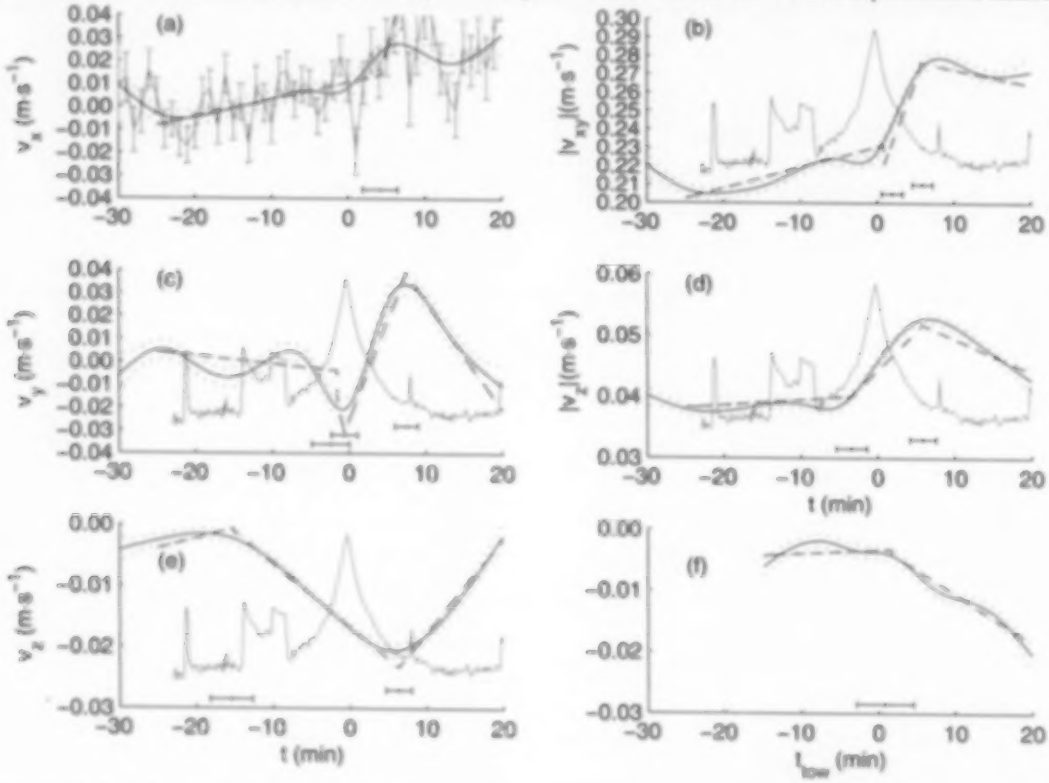
Fig. 5. The response variables as a function of time t (min) (*a*–*e*) and the diving v_z as a function of the t_{tow} variable (*f*). The solid lines and the dotted lines show the generalized additive model (GAM) spline curve and 95% confidence intervals, respectively. In (*a*), the gray curve is the running mean (RM) curve with standard error (± SE). In (*b*–*e*), the gray curve is the noise measurement transported from Fig. 1 based on that particular passing. The broken lines are the segmented linear regression curves. The horizontal bars in the lower parts of the panels indicate the estimated breakpoints with ±2 SEs from the segmented regression. Note that in the $|v_{xy}|$ plot (*b*), a discontinuous step is seen at the first breakpoint in the segmented regression. This is an artefact of the R-implementation method.



buoy (Fig. 5)). The initial response is diving, and the first breakpoint of the mean vertical component, v_z, occurs 15 min ($x_{bp} \sim$ 450 m) before the vessel passes the buoy (Fig. 5*e*). This occurs before any obvious change in vessel noise level. However, analysis of the diving response using the time variable t_{tow} reveals a clear breakpoint at $t_{tow} = 0.9 \pm 1.9$ (SE) min (i.e., at about the time the trawl starts running) (Fig. 5*f*). Note that $t_{tow} = 0$ is equivalent to the start-towing event (Fig. 1) where a steep decrease in vessel noise is seen. The absolute value of the vertical response, $|v_z|$, does not change before the vessel noise increases (Fig. 5*d*). This can be interpreted as the fish being reluctant to swim upwards in the pre-vessel zone and that the increased swimming speed does not occur before the vessel noise level increases. The time of the maximum $|v_z|$ occurs approximately 6 min after the vessel passing and does not correspond to the maximum vessel noise level.

After the initial diving response, the slope of the alongship directional response measured by v_x is significantly different from zero (Fig. 5*a*). This indicates a (weak) herding in front of the vessel, and the effect increases as the vessel approaches. The onset time for the herding effect is not detected by the segmented regression method, and the nonparametric plots suggest that there is no clear breakpoint in the pre-vessel zone. There seems to be a discontinuity (a negative step) in alongship swimming when the vessel is passing, detected by the RM curve (Fig. 5*a*). This is not seen in the GAM spline because of heavy smoothing. A negative step means that the fish changes direction from swimming with the vessel to swimming against the vessel. When using d_{bp} as an explanatory variable (not shown), this step occurred before $d_{bp} = 0$ (i.e., the fish turns towards the propeller before propeller passing). The positioning is quite accurate close to the origin of the coordinate system, and it seems that the fish reacts with a directional response towards the hull rather than towards the propeller.

The athwartship response is negative in terms of v_y as the vessel noise increases (Fig. 5*c*) (i.e., the fish responds by swimming towards the vessel). This effect is also detected by the segmented regression. After vessel passage this changes, and there is a strong directional herding away from the vessel path.

In general, the strongest response, both vertically and horizontally, is seen after vessel passing when the vessel noise decreases. For example, the highest diving speed occurs 6 min after vessel passing. In addition, the directional polarity of this response is stronger than that of the reaction towards the vessel. There are quite similar responses to the warp and (or) trawl in terms of d_w and d_n (Fig. 6). In general, the breakpoints de-

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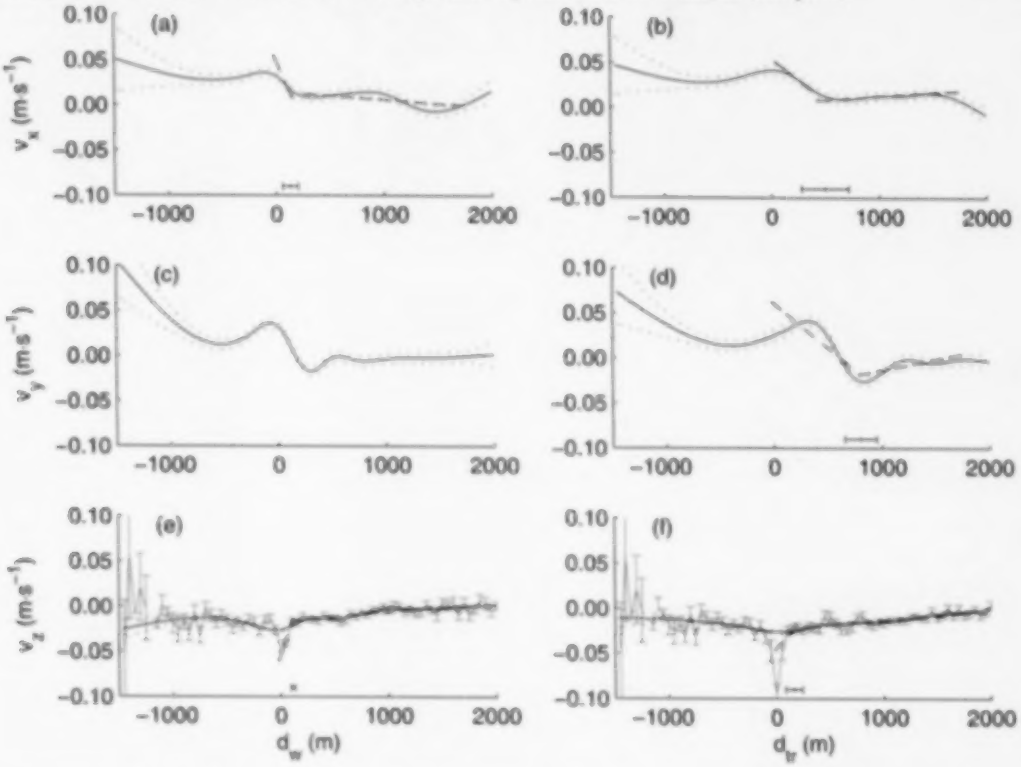
Fig. 6. The response variables v_x (*a* and *b*), v_y (*c* and *d*), v_z (*e* and *f*) as a function of the distance to the warp d_w and distance to the trawl doors d_{tr}. The solid lines and the dotted lines show the generalized additive model (GAM) spline curve and 95% confidence intervals, respectively. The horizontal bars in the lower parts of the panels indicate the estimated breakpoints with ±2 standard errors (SEs) from the segmented regression. In (*e*–*f*), the gray curve is the running mean (RM) curve with ±2 SEs. Note the steep response close to $d_w = 0$ m indicated by the broken lines for the segmented regression and the estimated breakpoints.



tected by the segmented regression method are located closer to $d_w = 0$ than to $d_{tr} = 0$, and the regression slopes are steeper near the warps. This is seen both for the vertical and horizontal speed (Figs. 6*b*, 6*d*). This suggests that the fish reacts rather sharply to the warps, especially seen in the diving reaction v_z (Fig. 6*e*), the vertical speed $|v_z|$ (not shown), and the alongship swimming v_x (Fig. 6*a*).

It is important to note that the directional response was not strong. The mean vertical velocity slowly decreased from 0 to ≈ −2 cm·s^{-1}, whereas the speed was an order of magnitude higher (Fig. 5), indicating a high degree of random swimming. If the (t, v_z) curve is integrated from the first reaction to the time when the trawl passes the buoy, it corresponds to a mean vertical displacement of ≈20 m. Similarly, the integrated alongship swimming over the span of the reaction indicates a mean alongship herding displacement of ≈17 m. The integrated athwarthship swimming over the duration of the fish reaction results in a mean horizontal herding displacement of only 4 m, but note that the sign is changing along the integration, thus reducing the apparent magnitude of the effect.

To further elucidate the observed reaction pattern, we provide a three-dimensional perspective of the response resolved in RM bins in depth and horizontal distance to the propeller (Fig. 7), including top, front, side, rear, and perspective view (Figs. 7*a*, 7*b*, 7*c*, 7*d*, and 7*e*, respectively). The size of the bins in depth and horizontal distance (d_{bp}) are 50 and 100 m, respectively, and the numbers of bins in depth and horizontal distance are four and ten, respectively. The cones of the figure are centred in each bin, and the depth is shown by different colour, where the upper to lower bins are shown as colours ranging from yellow to red. The response is not resolved in the athwartship direction, but is assumed to be equal on each side of the boat. The cones representing the reaction is therefore mirrored along the x–z plane. The apex of the cones is pointing in the direction of the response, and the height is proportional to the magnitude of the velocity vector. This representation gives a good visualization of the observed reaction pattern close to the vessel and gear.

The response to the vessel is similar to that detected by the two-dimensional plots (Figs. 5 and 6), but with the added dimension of depth. By simultaneously assessing the alongship and athwarthsip response (Figs. 7*a*, 7*b*), it may seem at first sight to correspond to the gradient of the typical butterfly sound pattern from vessels (Urick 1967, p. 273). However, at the bin of vessel passing, the response (Figs. 5*b* and 7*a*) indicates a reaction straight towards the vessel, at least in the upper layers of depth (yellow cones), thus not consistent with the butterfly pattern. The attraction component towards the vessel also occurs at the deep RM bins before and after the vessel passing

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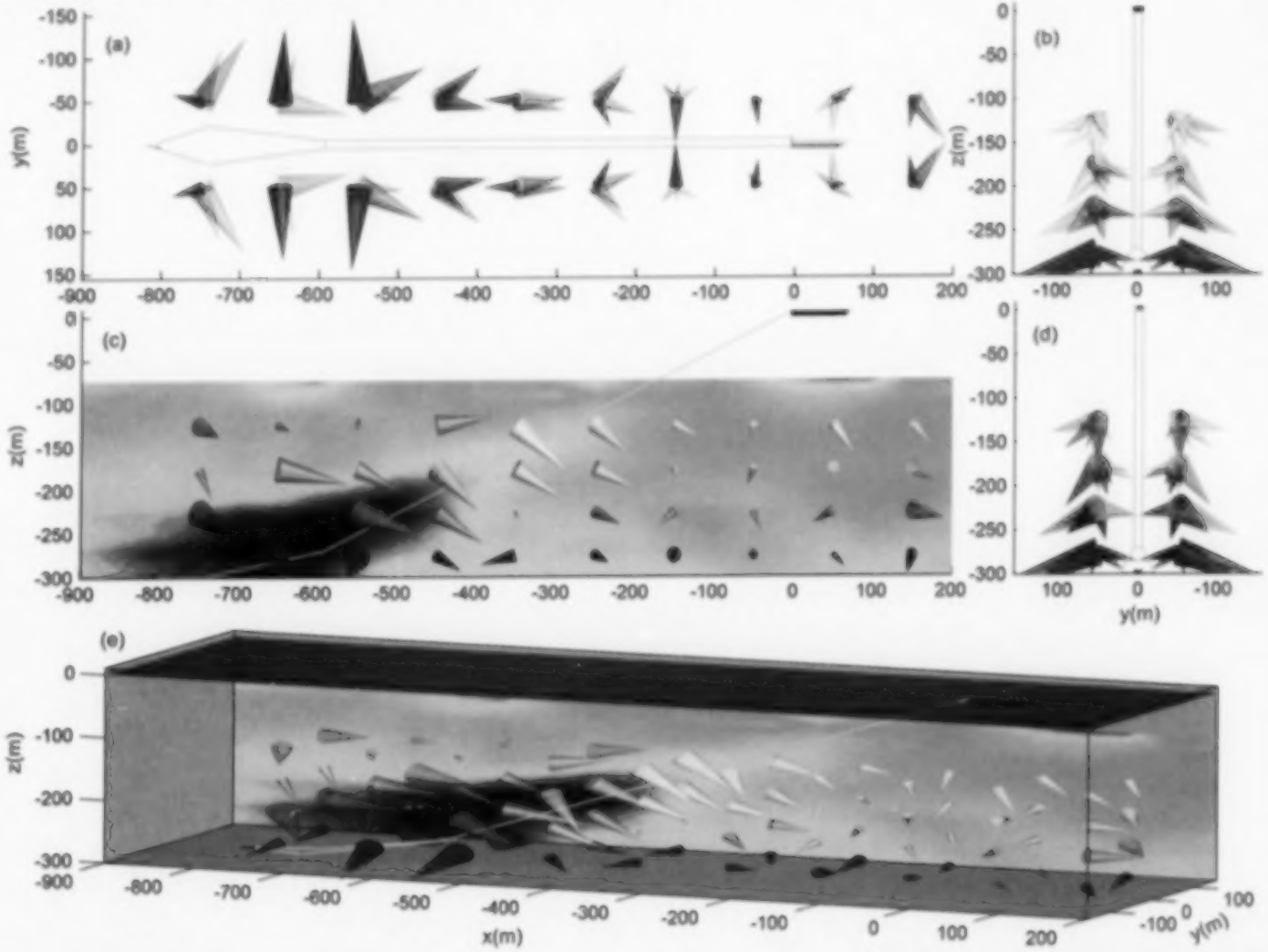
Fig. 7. The three-dimensional perspective of the directional response resolved in depth and the horizontal distance to the propeller d_{xy}: (*a*) the top view, (*b*) the front view, (*c*) the side view, (*d*) the rear view, and (*e*) shows the 3D perspective. The cones show the direction and the strength of $v = [v_x\ v_y\ v_z]$, and the colour of the cones indicate the depth bin, where red is the bottom bin and yellow is the surface bin. Gray cones indicate the bins above and after warps, where in general there are few tracks within each bin. The response is not resolved in the athwarthsip direction, and the vectors are mirrored along $y = 0$ for illustrative purposes. The swimming speed, $\sqrt{|v_x|^2 + |v_z|^2}$, is shown in the background of panels (*c*) and (*e*). The range is from ~ 0.04 to ~ 0.1 m·s^{-1}.



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bin (red cones at $d_{hp} = 150$ m and $d_{hp} = -50$ m), where the butterfly effect is reduced.

The response towards the warps and trawl reveals a strong alongship response in the pelagic region (Fig. 7, yellow cones) and a strong athwartship reaction closer to the bottom (Fig. 7, red cones). This reaction is dominantly away from the vessel path (i.e., the opposite response to that induced by the vessel). This indicates an alongship herding response away from the warp and an athwartship herding response away from the parts of the warps closer to the bottom and possibly to the doors. After the warp has passed, fewer fish are detected. This is visualized in the plot by colouring the cones in grey scale. The swimming speed is also increased in the vicinity of the warp (Figs. 7*c*, 7*e*, background plot). Note also the general, stronger, directional response towards the warps indicated by the larger size of the cones close to the warp.

We also categorized our data according to day–night, size, density, and year-to-year effects. No clear differences were found except that both the vertical and horizontal speeds seemed generally to be higher in daytime than at night and there was a relatively small year-to-year difference for the directional response. This is especially true of the alongship response, which seems to be somewhat different in nature, not only in strength. For the athwarthship response, the strength changed but otherwise it had similar features between years.

Discussion

The method and sources of errors

The first crucial step in obtaining tracks from a split-beam echo sounder is the processing of potential targets by the SED algorithm. This algorithm fails when the fish density is high, and it is therefore difficult to make inferences about density-dependent behaviour. It may be that the critical density for more uniform behaviour is too high for the SED to work properly. This may explain why no dependence on the fish density is apparent during the vessel passings. Lowering the transducer to reduce the distance to the fish may partly solve this problem, but it would also reduce the observation volume.

If the transducer rolls cyclically, the estimated horizontal speed will increase linearly with depth. The tracker is designed to estimate and correct for this movement. In addition, for long tracks covering the period of the transducer movement, the linear regression method will average out the roll effect to a large extent, and as explained in the Materials and method section, only tracks exceeding 15 registrations have been kept. Another potential source of error is the wake-induced tilt and roll movement of the transducer as the vessel passes. This would increase the apparent horizontal speed after the vessel passing. We believe that this is not affecting our results, based on the following argument: the increase in athwarthship and alongship swimming velocity is not related to an increase in the transducer tilt–roll because the velocity increase is directional, and the faster horizontal speed seems to occur close to the warps (i.e., not in the whole water column simultaneously). Performing the analysis using different current models and adding an artificial tilt angle to the transducer do not affect the results.

So far we have discussed the implications and data correction methods applicable to a rolling transducer. However, these methods only work for periodic movements. It could be that the transducer axis is tilted off the vertical. We ran the analysis with an added constant transducer tilt of 10°. The course of the reaction was not altered, except that the mean vertical velocity changed by -0.01 m·s^{-1}. We conclude that our results and findings are not sensitive to a biased tilt angle.

The measured velocities are combinations of water currents and active fish swimming. To analyse the swimming component, a very simple depth-dependent water-current estimation technique was applied to the horizontal velocities (see the Materials and method section). In addition, we tried a current estimate with no depth dependence. This was assumed equal to the mean of the measured horizontal fish speeds in the water column. It was found that the speed estimated from the raw data was higher, but the course of the reaction was similar. This indicates that our results and conclusions are not sensitive to the water currents. However, the currents may also influence the behaviour. It has been shown that cod may utilize the tidal stream for transport (Arnold et al. 1994), and Aglen et al. (1999) showed that when the current speed increased 50 m above the bottom, the fish moved closer to the bottom. Although we are mainly interested in the vessel-induced behaviour in this paper, these effects should be taken into account when interpreting the abundance estimates from trawl surveys.

Knowledge of the positioning of the gear and vessel is discussed in Handegard (2004, pp. 87–91). In general, the accuracy is poorer, especially for the athwarthship positioning, at greater distances from the vessel. This is because the ITI measures the angle and distance to the headline of the trawl relative to the vessel. If the angular error is constant, the athwarthship positioning error increases with distance. A rather simplified model of the trawl geometry is used, but owing to the positioning error, we argue that this is adequate for our system.

The fish reaction

The noise level from the vessel is known to influence fish behaviour, and the noise level is variable between vessels and at different vessel speeds and loads (Mitson 1993; Mitson and Knudsen 2003). To overcome this problem, much effort has been made to make research vessels quieter, and the International Council for Exploration of the Sea (ICES) has made recommendations for the noise level during free running for new vessels (Mitson 1995, his fig. 22). The (former) *G.O. Sars* is a very noisy vessel (Mitson 1995, his fig. 13), but a major finding of this study was that vessel noise does not seem to explain the main features of the observed response. On the contrary, the fish seems to respond first when there is a net reduction of noise energy emitted from the vessel (in the frequency band between 40 and 300 Hz) at the start-trawling event. One explanation could be that the fish reacts to a change in noise level, even if it is a reduction. But if the fish reacts to a change, it should also react to the increase in the noise level 5–6 min before the start-towing event. Another possible explanation is that the diving response is triggered when the trawl doors hit the bottom. This may cause low frequency thuds that trigger the reaction.

It is important to note that the noise measurements were conducted in a fjord, where the noise propagation could be different from that in the open sea. This may be due to a different vertical sound speed profile, and consequently different refraction, or reflections from the boundaries of the fjord. The implication of

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refraction is more prominent farther away from the source, and the measured noise level between setting the trawl and passing the buoy may not be entirely representative for the ocean experiments. However, the nature of the main increase in noise level would be similar. In addition, conductivity–temperature–depth stations at the noise measurement site and the experimental site showed similar vertical sound speed profiles.

Whether it is the sound of the trawl doors or the abrupt change in vessel noise that motivates the fish, it is evident that the handling of the boat when setting the trawl affects fish behaviour. The handling technique may be different from one skipper to another, and it is important to standardize not only the towing time and gear geometry but also the manner of controlling the trawl during the set.

Two or three minutes before vessel passing, the fish moves towards the vessel path. This is a rather counter-intuitive behavioural pattern. One explanation could be the properties of the sound field around the vessel. The propeller noise is not radiated uniformly in all directions. It is less in the fore and aft directions, probably owing to screening by the hull and the wake, respectively (Urick 1967, p. 273). This feature is used to explain the horizontal herding of pelagic species, such as herring (Misund et al. 1996) and anchovy and sardines (Soria et al. 1996), into the path of the vessel. If this is the cause, one should expect the effect to end by the time of propeller passing. The effect is reduced but not terminated at this time, and there seems to be an attraction component towards the vessel. One possible explanation is that the fish experiences the vessel as a fish-aggregating device (FAD) (S. Kaartvedt, Department of Biology, University of Oslo, P.O. Box 1064 Blindern, 0316 Oslo, Norway, personal communication) (i.e., the fish is actually attracted towards the vessel in a similar way as FADs). Previous evidence on this phenomenon is somewhat conflicting. The observed attraction towards the vessel is in contradiction to Ona and Godø (1990) and Nunnallee (1991), who reported a density draining phenomenon in front of an approaching trawler in studies of haddock and Pacific whiting (*Merluccius productus*), respectively. Ona and Godø (1990) also found a significantly lower acoustic density during trawling as compared with a free-running vessel, which they attributed to fish moving horizontally out of the vessel path. On the other hand, recent studies have shown that acoustic registrations on R/V *G.O. Sars* are in fact 10%–15% higher while trawling than when free running (V. Hjellvik, CATEFA Project, Institute of Marine Research, P.O. Box 1870, 5817 Bergen, Norway, unpublished data), whereas for the other vessels there were no significant differences between trawling and free running. This supports our findings but may indicate that it is a vessel-specific response, and not fish-specific as in the FAD hypothesis.

Reactions to the trawl warps have been reported earlier for Pacific whiting (Nunnallee 1991), but the strength and directionality of the effect were not assessed. Our analysis shows that the warps induce the strongest and sharpest reaction of the fish. The vibration of the warps generated sound with spectral peaks at 7 and 14 Hz. These frequencies are within the hearing range of cod (Sand and Karlsen 1986), and fish reaction to detectable sound at such low frequencies is believed to be strong. Enger et al. (1993) found that juvenile salmon reacted to a 10-Hz noise source, but not to one at 150 Hz. Cod are also able to sense the direction to the sound source (Schuijf and Buwalda 1975; Schuijf 1975). We did not measure the amplitude of the tones generated by the warps, but we had hoped to detect the 7- and 14-Hz peak warp vibration frequencies in the noise measurements and then correlate these with the measured reaction. We were unable to do so, possibly because other noise sources were too strong at these low frequencies. The ambient noise due to the turbulence in deep ocean currents is high at these frequencies (Urick 1967, page 164). The tones are emitted continuously, but the warp tension (and therefore the tone frequency) may vary. It has been suggested that the fish reaction may be motivated by frequency shifts in the warp vibrations (O.R. Godø, Institute of Marine Research, P.O. Box 1870, 5817 Bergen, Norway, personal communication). Unfortunately, our warp measurements were not resolved in time. Although we were not able to detect the warps from our recordings, the question still remains whether the fish is able to do so.

Another explanation of the strong response towards the warps is light, either from the surface or induced by bioluminescence. Video and flash photo observations of fish herded by the trawl doors and the trawl sweeps under different light conditions have been conducted (Wardle 1993). If the light conditions were adequate, the fish kept visual contact when avoiding the trawl door and the trailing sand cloud. This behaviour was closely related to the light level. We did analyse diel variation in the response but found little effect of time of day. Light transmitted from the surface can hardly be the explanation of our observed strong response. Another possible stimulus is bioluminescence. Indeed, the vibrating warps may generate bioluminescence, and a directional and variable response (also with year) could be expected because the bioluminescent intensity may vary with the water turbidity and the abundance of the organisms concerned. Since the vertical reaction is stable while the horizontal response varies from year to year, it could be that the horizontal directional response is predominantly triggered by bioluminescence, while the vertical response depends on the warp tones. To reach more definite conclusions about this problem, we would have to more closely investigate the warp and trawl sounds and the amount of bioluminescence in the area.

Explaining the fish reactions seen in our experiments only in terms of each stimulus separately is an oversimplification of the problem. Certainly the fish does not react to light, vessel noise, or warp tones separately, and it is probably not correct to view the response as a function of each response and explanatory variable separately. A natural approach would be to explain the response using several explanatory variables simultaneously. We tried to fit a GAM to the response variables with two or more explanatory variables, but this approach failed. The domain was not spanned properly by the explanatory variables, because of the fixed relationship between d_{hp}, d_{tr}, and to some extent d_o. Other response and explanatory variables were implemented, including the distance to the propeller and the horizontal directional swimming away from the different stimuli. These studies did not provide any new information. Consequently, the reactions were analysed in terms of a single response and explanatory variable separately.

Another simplification is the assumption that the response can be explained by the initiating stimuli alone. The internal state of fish determines the reaction thresholds of fish. If the fish changes vigilance after the approach and passing of the vessel, and as a consequence displays a stronger reaction towards the

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warps, we would falsely conclude that the vessel had no impact on the reaction.

The gross features of the fish reactions are the same from year to year and from one location to another, and this is particularly true of the vertical velocity. This indicates that the measured velocity changes are largely representative of the reactions of gadoids at the time of year and in the area investigated. Thus, the findings have clear implications for the combined trawl and acoustic survey conducted in Norwegian waters during the winter (Jakobsen et al. 1997). For example, it is possible to fit the data to models for estimating parameters relevant for the trawl survey index. Some initial work has been published on this (Handegard and Tjøstheim 2004). However, the results are also of interest for the trawl capture process, and thus for trawl surveys in general. It constitutes a framework for understanding how a fish may react to an approaching vessel and suggests some implications in the way we treat the trawl as a sampling tool. For example, focus has traditionally been on selection processes close to the trawl, like herding of the sweeps (Engås and Godø 1989*a*), doors (Wardle 1993), bottom gear selection (Engås and Godø 1989*b*), and selection processes within the trawl (Marlen 2003). Little attention has been paid to the behaviour, and thus possible selection effects, before the appearance of the trawl doors. It has been mentioned by some (Dickson 1993; Graham et al. 2004) but not really addressed so far. Our results indicate that there may be a large-scale selection before the appearance of the trawl doors, and designing new sampling gear while disregarding these processes may be erroneous.

Some questions about fish behaviour were posed in the Introduction, and we are now in the position to shed some light on them. We are able to conclude that the fish reaction, in our case, were primarily not explained by the gradual increase in vessel noise as the vessel approached. The start-trawling event seemed to be the trigger that initiated the diving response. Whether this is due to trawl noise or changes in the emitted vessel noise is not clear. The induced reaction as a response to the warps and gear is stronger than that of the vessel. In addition, this response has a strong directionality. The hypothesis that a fish swims away from the vessel as a response to vessel noise seems to be false, at least in our case. The fish actually swam towards the vessel at the time of vessel passing. We have pointed to several possible explanations for this, including that it may be a vessel-specific effect. An important motivation for this study was to evaluate possible consequences for the bottom trawl survey. In this respect, the observed strong directional herding from the warps is an effect that may induce selection processes that have until now been disregarded.

Acknowledgements

We first thank Kathrine Michalsen for valuable feedback during the work. We also thank Atle Totland, Ingvald Svellingen, and the crew on R/V *G.O. Sars* for skilful handling of the buoy system, and Jan Tore Øvredal for help with the noise recordings and analysis. The Norwegian Research Council financially supported the work (Project 133426/120). This work is a part of the Dr. Scient. thesis by Nils Olav Handegard, and we thank the committee, Jens M. Hovem, Stein Kaartvedt, and Trygve Nilsen, for valuable feedback and criticism during the defence of the thesis.

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refraction is more prominent farther away from the source, and the measured noise level between setting the trawl and passing the buoy may not be entirely representative for the ocean experiments. However, the nature of the main increase in noise level would be similar. In addition, conductivity–temperature–depth stations at the noise measurement site and the experimental site showed similar vertical sound speed profiles.

Whether it is the sound of the trawl doors or the abrupt change in vessel noise that motivates the fish, it is evident that the handling of the boat when setting the trawl affects fish behaviour. The handling technique may be different from one skipper to another, and it is important to standardize not only the towing time and gear geometry but also the manner of controlling the trawl during the set.

Two or three minutes before vessel passing, the fish moves towards the vessel path. This is a rather counter-intuitive behavioural pattern. One explanation could be the properties of the sound field around the vessel. The propeller noise is not radiated uniformly in all directions. It is less in the fore and aft directions, probably owing to screening by the hull and the wake, respectively (Urick 1967, p. 273). This feature is used to explain the horizontal herding of pelagic species, such as herring (Misund et al. 1996) and anchovy and sardines (Soria et al. 1996), into the path of the vessel. If this is the cause, one should expect the effect to end by the time of propeller passing. The effect is reduced but not terminated at this time, and there seems to be an attraction component towards the vessel. One possible explanation is that the fish experiences the vessel as a fish-aggregating device (FAD) (S. Kaartvedt, Department of Biology, University of Oslo, P.O. Box 1064 Blindern, 0316 Oslo, Norway, personal communication) (i.e., the fish is actually attracted towards the vessel in a similar way as FADs). Previous evidence on this phenomenon is somewhat conflicting. The observed attraction towards the vessel is in contradiction to Ona and Godø (1990) and Nunnallee (1991), who reported a density draining phenomenon in front of an approaching trawler in studies of haddock and Pacific whiting (*Merluccius productus*), respectively. Ona and Godø (1990) also found a significantly lower acoustic density during trawling as compared with a free-running vessel, which they attributed to fish moving horizontally out of the vessel path. On the other hand, recent studies have shown that acoustic registrations on R/V *G.O. Sars* are in fact 10%–15% higher while trawling than when free running (V. Hjellvik, CATEFA Project, Institute of Marine Research, P.O. Box 1870, 5817 Bergen, Norway, unpublished data), whereas for the other vessels there were no significant differences between trawling and free running. This supports our findings but may indicate that it is a vessel-specific response, and not fish-specific as in the FAD hypothesis.

Reactions to the trawl warps have been reported earlier for Pacific whiting (Nunnallee 1991), but the strength and directionality of the effect were not assessed. Our analysis shows that the warps induce the strongest and sharpest reaction of the fish. The vibration of the warps generated sound with spectral peaks at 7 and 14 Hz. These frequencies are within the hearing range of cod (Sand and Karlsen 1986), and fish reaction to detectable sound at such low frequencies is believed to be strong. Enger et al. (1993) found that juvenile salmon reacted to a 10-Hz noise source, but not to one at 150 Hz. Cod are also able to sense the direction to the sound source (Schuijf and Buwalda 1975; Schuijf 1975). We did not measure the amplitude of the tones generated by the warps, but we had hoped to detect the 7- and 14-Hz peak warp vibration frequencies in the noise measurements and then correlate these with the measured reaction. We were unable to do so, possibly because other noise sources were too strong at these low frequencies. The ambient noise due to the turbulence in deep ocean currents is high at these frequencies (Urick 1967, page 164). The tones are emitted continuously, but the warp tension (and therefore the tone frequency) may vary. It has been suggested that the fish reaction may be motivated by frequency shifts in the warp vibrations (O.R. Godø, Institute of Marine Research, P.O. Box 1870, 5817 Bergen, Norway, personal communication). Unfortunately, our warp measurements were not resolved in time. Although we were not able to detect the warps from our recordings, the question still remains whether the fish is able to do so.

Another explanation of the strong response towards the warps is light, either from the surface or induced by bioluminescence. Video and flash photo observations of fish herded by the trawl doors and the trawl sweeps under different light conditions have been conducted (Wardle 1993). If the light conditions were adequate, the fish kept visual contact when avoiding the trawl door and the trailing sand cloud. This behaviour was closely related to the light level. We did analyse diel variation in the response but found little effect of time of day. Light transmitted from the surface can hardly be the explanation of our observed strong response. Another possible stimulus is bioluminescence. Indeed, the vibrating warps may generate bioluminescence, and a directional and variable response (also with year) could be expected because the bioluminescent intensity may vary with the water turbidity and the abundance of the organisms concerned. Since the vertical reaction is stable while the horizontal response varies from year to year, it could be that the horizontal directional response is predominantly triggered by bioluminescence, while the vertical response depends on the warp tones. To reach more definite conclusions about this problem, we would have to more closely investigate the warp and trawl sounds and the amount of bioluminescence in the area.

Explaining the fish reactions seen in our experiments only in terms of each stimulus separately is an oversimplification of the problem. Certainly the fish does not react to light, vessel noise, or warp tones separately, and it is probably not correct to view the response as a function of each response and explanatory variable separately. A natural approach would be to explain the response using several explanatory variables simultaneously. We tried to fit a GAM to the response variables with two or more explanatory variables, but this approach failed. The domain was not spanned properly by the explanatory variables, because of the fixed relationship between d_{hp}, d_{tr}, and to some extent d_{vp}. Other response and explanatory variables were implemented, including the distance to the propeller and the horizontal directional swimming away from the different stimuli. These studies did not provide any new information. Consequently, the reactions were analysed in terms of a single response and explanatory variable separately.

Another simplification is the assumption that the response can be explained by the initiating stimuli alone. The internal state of fish determines the reaction thresholds of fish. If the fish changes vigilance after the approach and passing of the vessel, and as a consequence displays a stronger reaction towards the

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warps, we would falsely conclude that the vessel had no impact on the reaction.

The gross features of the fish reactions are the same from year to year and from one location to another, and this is particularly true of the vertical velocity. This indicates that the measured velocity changes are largely representative of the reactions of gadoids at the time of year and in the area investigated. Thus, the findings have clear implications for the combined trawl and acoustic survey conducted in Norwegian waters during the winter (Jakobsen et al. 1997). For example, it is possible to fit the data to models for estimating parameters relevant for the trawl survey index. Some initial work has been published on this (Handegard and Tjøstheim 2004). However, the results are also of interest for the trawl capture process, and thus for trawl surveys in general. It constitutes a framework for understanding how a fish may react to an approaching vessel and suggests some implications in the way we treat the trawl as a sampling tool. For example, focus has traditionally been on selection processes close to the trawl, like herding of the sweeps (Engås and Godø 1989*a*), doors (Wardle 1993), bottom gear selection (Engås and Godø 1989*b*), and selection processes within the trawl (Marlen 2003). Little attention has been paid to the behaviour, and thus possible selection effects, before the appearance of the trawl doors. It has been mentioned by some (Dickson 1993; Graham et al. 2004) but not really addressed so far. Our results indicate that there may be a large-scale selection before the appearance of the trawl doors, and designing new sampling gear while disregarding these processes may be erroneous.

Some questions about fish behaviour were posed in the Introduction, and we are now in the position to shed some light on them. We are able to conclude that the fish reaction, in our case, were primarily not explained by the gradual increase in vessel noise as the vessel approached. The start-trawling event seemed to be the trigger that initiated the diving response. Whether this is due to trawl noise or changes in the emitted vessel noise is not clear. The induced reaction as a response to the warps and gear is stronger than that of the vessel. In addition, this response has a strong directionality. The hypothesis that a fish swims away from the vessel as a response to vessel noise seems to be false, at least in our case. The fish actually swam towards the vessel at the time of vessel passing. We have pointed to several possible explanations for this, including that it may be a vessel-specific effect. An important motivation for this study was to evaluate possible consequences for the bottom trawl survey. In this respect, the observed strong directional herding from the warps is an effect that may induce selection processes that have until now been disregarded.

Acknowledgements

We first thank Kathrine Michalsen for valuable feedback during the work. We also thank Atle Totland, Ingvald Svellingen, and the crew on R/V *G.O. Sars* for skilful handling of the buoy system, and Jan Tore Øvredal for help with the noise recordings and analysis. The Norwegian Research Council financially supported the work (Project 133426/120). This work is a part of the Dr. Scient. thesis by Nils Olav Handegard, and we thank the committee, Jens M. Hovem, Stein Kaartvedt, and Trygve Nilsen, for valuable feedback and criticism during the defence of the thesis.

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Appendix A. Fitting the segmented regression model.

The segmented regression line model for v_z with breakpoints ψ_1, ψ_2, etc., is given by

$$v_z = \alpha t + \sum_i \beta_i (t - \psi_i) I(t > \psi_i)$$

where I is an indicator variable, α is the slope of the line segment prior to ψ_1, and β_1 is the difference in slope parameter for $t > \psi_1$ and $t < \psi_2$. If more than one breakpoint is searched, β_2 is the next change in slope, continuing until all the breakpoints are determined. When using the positions as explanatory variables, t is replaced by d, and for the other responses, v_z is replaced with the desired response variable. Note that our interest is primarily in the change points ψ_i rather than the regression parameters α and β_i. The regression model is nonlinear with respect to ψ_i. Linearization of the model around the estimated breakpoint $\hat{\psi}_i^{(k-1)}$, obtained at stage $k-1$ of the iterative procedure, yields

$$v_z = \alpha t + \sum_i \beta_i [t - \hat{\psi}_i^{(k-1)}] I[t > \hat{\psi}_i^{(k-1)}] + \sum_i \gamma_i I[t > \hat{\psi}_i^{(k-1)}]$$

where $\gamma_i = \beta_i [\psi_i - \psi_i^{(k-1)}]$. The problem is now reduced to a multiple linear regression. It is seen that for v_z the breakpoints can be expected around $t = -15$ and $t = 5$ min (Fig. 5*e*), and these are chosen as initial estimates $\psi_1^{(0)} = -15$ and $\psi_2^{(0)} = 5$, respectively. Every iteration yields least-squares estimates of $\hat{\alpha}_i^{(k-1)}$, $\hat{\beta}_i^{(k-1)}$, $\hat{\gamma}_i^{(k-1)}$, and subsequently $\hat{\psi}_i^{(k)} = [\hat{\gamma}_k^{(k-1)} / \hat{\beta}_k^{(k-1)}] + \hat{\psi}_i^{(k-1)}$. The iteration procedure terminates when $\hat{\psi}_i^{(k)} - \hat{\psi}_i^{(k-1)} = [\hat{\gamma}_i^{(k-1)} / \hat{\beta}_i^{(k-1)}]$ is deemed sufficiently small, at which stage we take $\hat{\alpha}_i = \hat{\alpha}_i^{(k-1)}$, $\hat{\beta}_i = \hat{\beta}_i^{(k-1)}$, and $\hat{\psi}_i = \hat{\psi}_i^{(k)}$. Note that this method assumes that change points exist, such that $\beta_i > 0$. We are not concerned with testing for the existence of change points.

Standard errors for the estimates can be found by bootstrapping, but following (Muggeo 2003) we chose to be guided by asymptotic analysis. The errors of $\hat{\alpha}_i$ and $\hat{\beta}_i$ are obtained by maximum likelihood and that of $\hat{\psi}_i$ by the delta method based on $\hat{\psi}_i = (\hat{\gamma}_i / \hat{\beta}_i) + \psi_i$. It should be noted that these formulae require a large number of tracks in each linear segment to obtain the derived asymptotic distribution as a reasonable approximation. This means that the p values of the estimated regression parameters should be interpreted with caution. Typically, the number of tracks for each regression segment is between 1000 and 15 000, which would be sufficient, but we also have segments with fewer observations.

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ERRATUM / ERRATUM

Erratum: A statistical model for in-season forecasts of sockeye salmon (*Oncorhynchus nerka*) returns to the Bristol Bay districts of Alaska

Saang-Yoon Hyun, Ray Hilborn, James J. Anderson, and Billy Ernst

Ref.: Can. J. Fish. Aquat. Sci. **62**: 1665–1680 (2005).

On p. 1671, in the second line before eq. 12, the right-hand parenthesis appeared as a subscript. The line should read "four-age return sizes per stock ($\overline{r}_{s,1}, \overline{r}_{s,2}, \overline{r}_{s,3}, \overline{r}_{s,4}$) in eq. 11".

On p. 1672, in the first equation, the definition sign (≡) should be an equals sign (=). The corrected equation is as follows:

$$L(\overline{\mathbf{r}}_1, \overline{\mathbf{r}}_2, \ldots, \overline{\mathbf{r}}_5) = \prod_{s=1}^{5} L(\overline{\mathbf{r}}_s).$$

On p. 1680, in Table A3, "($\times 10^3$)" should be removed after the headings "Forecast" and "Actual run". For clarity, the corrected table is reprinted here.

Table A3. Relative errors of University of Washington Alaska Salmon Program preseason forecasts of sockeye salmon (*Oncorhynchus nerka*) returns to the Bristol Bay districts of Alaska.

Year	Stock	Forecast	Actual run	Error (%)
1999	N–K	17 700 000	16 763 000	5.6
	E	7 700 000	9 150 000	–15.8
	U	2 700 000	3 919 000	–31.1
	N	6 640 000	8 484 000	–21.7
	T	380 000	504 000	–24.6
2000	N–K	14 200 000	7 878 043	80.2
	E	11 400 000	8 137 718	40.1
	U	4 900 000	2 128 114	130.3
	N	6 200 000	8 545 112	–27.4
	T	940 000	1 105 470	–15.0
2001	N–K	6 800 000	8 170 269	–16.8
	E	5 300 000	3 830 735	38.4
	U	1 400 000	1 309 431	6.9
	N	7 800 000	7 473 115	4.4
	T	480 000	1 108 133	–56.7

Note: Negative and positive values of errors indicate under- and over-forecasts. N–K, Naknek–Kvichak; E, Egegik; U, Ugashik; N, Nushagak; T, Togiak.

Received 25 September 2003. Accepted 8 February 2005. Published on the NRC Research Press Web site at http://cjfas.nrc.ca on 24 September 2005.
J17764E

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 doi: 10.1139/F05-186 © 2005 NRC Canada

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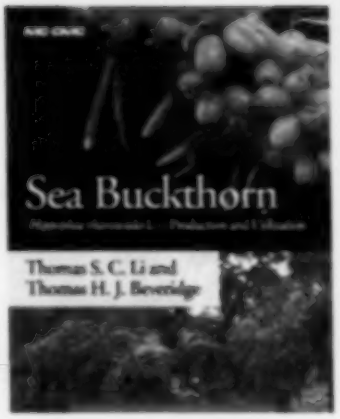

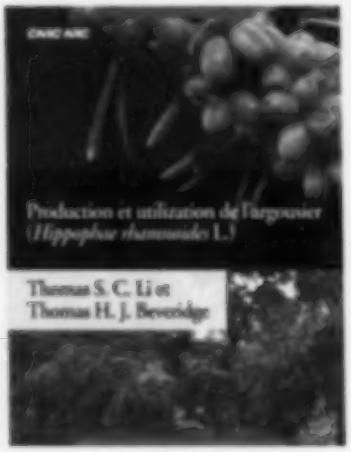

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Canadian Journal of Fisheries and Aquatic Sciences

Volume 62, Number 10, October 2005

Journal canadien des sciences halieutiques et aquatiques

Volume 62, numéro 10, octobre 2005

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NRC Reprographic Services / Service de reprographie CNRC
Page production by / Mise en page par
NRC Research Press / Presses scientifiques du CNRC

0706-652X(200510)62:10;1-Y